THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY
CHENNAI –TAMIL NADU

A STUDY
OF
'NON ALCOHOLIC FATTY LIVER DISEASE
IN
TYPE 2 DIABETES MELLITUS'

SUBMITTED FOR THE MD DEGREE EXAMINATION

BRANCH I

(GENERAL MEDICINE)

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AIM OF THIS STUDY

1.To find out the Prevalence and General Characteristics of Non Alcoholic Fatty Liver Disease in persons with Type 2 Diabetes Mellitus attending outpatient clinic in Thanjavur Medical College.

2.To asses the different clinical presentations of Non Alcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus Patients.

3.To asses the relationship between Body Mass Index and Non Alcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus.

4. To correlate the results of Liver Function Tests with Ultrasonographic evidence of fatty liver in Type 2 Diabetes Mellitus.

5. To correlate the results of fasting Lipid Profile with Ultrasonographic evidence of fatty liver in Type 2 Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus is a common metabolic disorder that affects a large number of people worldwide, the diabetic population is ever growing and it has now reached enormous proportions. Diabetes mellitus affects almost all systems in the body and it causes considerable morbidity and mortality. The liver is the hub of most of the metabolic activities and it plays a vital role in the metabolism of carbohydrates, proteins and lipids. Affection of liver in diabetes has been studied by many investigators. Of particular interest is an entity called Non alcoholic fatty liver disease (NAFLD) which occurs in a significant proportion of people who do not consume alcohol. Diabetes Mellitus, Hyperlipidemia and Obesity have been implicated as potential causes for the development of non alcoholic fatty liver disease and now newer risk factors have also been proposed. Nonalcoholic Fatty Liver Disease is a broader term that encompasses a spectrum including patients with simple steatosis, steatohepatitis that can progress to cirrhosis liver and even hepatocellular carcinoma. A plethora of case series of Non Alcoholic Fatty Liver Disease have been reported over the past few years but whether this indicates a true increase in prevalence or simply an increased awareness of this disorder is unclear. There have been a lot of studies done on Non Alcoholic Fatty Liver Disease in many centers around the world and a few

centers in India. No such study has been conducted till date in this part of our country.

Thanjavur Medical College located in Tamilnadu caters to the medical needs of a large diabetic population of about 3 districts; many cases of diabetes mellitus also have chronic liver disease, some of them do not have a history of significant alcohol consumption, so we thought that these cases might represent a sample of what is called cryptogenic cirrhosis.

A significant proportion of patients previously thought to have cryptogenic cirrhosis share many of the clinical and demographic features of nonalcoholic fatty liver disease, suggesting that the etiology of their cirrhosis may be unrecognized nonalcoholic fatty liver disease.

So we conducted this study to evaluate the prevalence and general characteristics of Nonalcoholic Fatty Liver Disease in type 2 diabetics with a motive to provide some information that might be useful for future reference and to evaluate the impact of this disease on persons belonging to this geographical region.

MATERIALS AND METHODS

Inclusion criteria

Patients who were diagnosed to have Type 2 Diabetes Mellitus, for more than 3 years duration, belonging to both sexes and with age of more than 40 years attending Diabetology Out - Patient Department of Thanjavur Medical College were included in the study.

Exclusion criteria

Patients with history of alcohol consumption for any duration of time were excluded.

Persons with previous history of Jaundice, Ascites, and signs of Liver cell failure were excluded.

Persons who tested positive for Hepatitis B serology by Elisa or by card test were excluded.

Patients with history of intake of Drugs, Methotrexate, Amiodarone, Glucocorticoids, Synthetic Estrogens, Glitazones, Nucleoside Analogues (ddI, AZT) were excluded.

Persons with history of major abdominal surgeries were excluded.

Persons with history of Chronic Renal Failure and Severe Ischemic Heart Disease were excluded from the study.

Patients with a history of Ketoacidosis or with a history prolonged treatment with insulin were excluded.

The Study Population was derived from the patients attending the Diabetology Outpatient Department of Thanjavur Medical College from April 2004 to January 2006.

A detailed history was taken regarding the Duration of Diabetes, Symptoms pertaining to the Hepatobiliary System

History of medications was obtained in detail.

History of alcohol consumption was recorded and any person with history of alcohol use was excluded from the study population.

Any history of previous abdominal surgeries such as Jejuno Ileal Bypass, Gastrectomy was recorded.

Women were enquired about oral contraceptive or hormonal use.

A detailed Clinical Examination of all systems was made and signs of Liver Cell Failure, Organomegaly, Ascites were looked for.

The patient's Height & Weight were recorded & Body Mass Index was calculated.

BMI was defined as weight in kilograms divided by (height in meters) [2.]

Patients were classified according to BMI as follows:

Underweight: BMI < 18.5 kg/m^2

Normal weight: BMI 18.5 to 24.9 kg/m^2

Overweight: BMI 25 to 29.9 kg/m^2

Obese: BMI > 30 kg/m^2

Blood Pressure measurements were taken.

THE LABORATORY INVESTIGATIONS DONE INCLUDED:

A Complete Blood Count.

Urine for Albumin Sugar and Deposits.

Blood Sugar (Random, Fasting & Post Prandial).

Blood Urea and Serum Creatinine.

Serum Electrolytes (Sodium & Potassium).

LIVER FUNCTION TESTS

SGOT (Normal value 5 to 35 IU/L)

SGPT (Normal value 5 to 35 IU/L)

Serum Alkaline Phospatase (Normal value 60 to 170 IU/L)

Serum Total Bilurubin (Normal value < 1 mg/dl)

Serum Total Proteins

FASTING LIPID PROFILE

The fasting lipid profile was done after a minimum of 12 hours of overnight fasting and the following tests were done

Serum Total Cholesterol

Serum Triglycerides (TGL)

Serum High Density Lipoprotein (HDL)

Serum Low Density Lipoprotein (LDL) was calculated using the Friedwald formula:

LDL-C = Total Cholesterol - HDL C – (Triglyceride/ 5).

All the Biochemical Investigations were done using Auto-analyzer technique with the ERBA XL 300 AUTOANALYZER.

Ultrasonogram of Abdomen was done with particular focus on the liver. Liver biopsy was done in a few selected cases considering the invasive nature of this procedure if necessary with a fully informed and detailed consent.

The presence of diabetes was defined according to the WHO CRITERIA[99] as:

Symptoms of diabetes, plus Random Blood Glucose concentration more than 200 mg/dl.

Fasting plasma glucose more than 126 mg/dl. (Fasting is defined as No caloric intake for at least 8h)

Two hour plasma glucose more than 200 mg/dl during an oral Glucose tolerance test. (This test should be done using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water)

Type 2 DM subjects were defined as those with previous physician-diagnosed diabetes in whom hyperglycemia had been controlled for one year or more with oral hypoglycemic agents and diet, with absence of history of ketoacidosis initially, or during the course of the disease [103,104].

IMAGING STUDY:

An Ultrasound scan of the abdomen was done with particular focus on the liver

Steatosis was defined as the presence of an ultrasonographic pattern consistent with "**BRIGHT LIVER**," with evident ultrasonographic contrast between hepatic and renal parenchyma, vessel blurring, focal sparing, and narrowing of the lumen of the hepatic veins, according to international guidelines[45,106] .Previous studies indicated that ultrasonography can detect and quantitate hepatic fat accumulation with an accuracy similar to that of computed tomography and liver biopsy.[107]

The upper limit of normal liver size was 15 cm in the longitudinal plane; any measurement above this was considered hepatomegaly. Mild hepatomegaly was defined as liver size >15-18 cm in the longitudinal plane.

The presence of steatosis was graded from mild to severe and for calculation purposes all grades were taken as positive fatty liver.

All the images were reviewed by another radiologist to minimise observer errors.

The L & T Ultrasound machine used had a 3.5 mhz probe.

Liver biopsy was done using the Menghini technique after adopting the standard protocols and after a fully informed consent from the patient. A platelet count was done, coagulation profile was done and vitamin K was given as intramuscular injection for 3 days prior to the procedure.

STATISTICAL ANALYSIS

Statistical analysis of the data obtained from the study was done using the **'z' test** or **'normal' test** to compare the mean values of two groups of participants. The **chi- square test** was used to compare the prevalence between two groups. The calculations were done for 5% level of significance.(P=0.05)

REVIEW OF LITERATURE

The incidence of diabetes mellitus is ever increasing and Diabetes mellitus is the common cause of increased morbidity and mortality. Diabetes mellitus affects many organs and systems in our body.

The liver plays a pivotal role in the metabolism of carbohydrates and lipids; affection of liver is common in diabetes. Sometimes liver disease may give rise to abnormalities in glucose homeostasis, and finally certain diseases of liver might be present coincidentally with diabetes.[1]

LIVER DISEASE IN DIABETES MELLITUS

1. Liver disease occurring as a consequence of diabetes mellitus

- **Glycogen deposition**
- **Steatosis and nonalcoholic steatohepatitis (NASH)**
- **Fibrosis and cirrhosis**
- **Biliary disease, cholelithiasis, cholecystitis**
- **Complications of therapy of diabetes (cholestatic and necroinflammatory)**

2. Abnormalities of glucose homeostasis occurring as a complication of liver disease can be present in

- **Hepatitis**
- **Cirrhosis**
- **Hepatocellular carcinoma**
- **Fulminant hepatic failure**
- **Post orthotopic liver transplantation**

3. Liver disease occurring coincidentally with diabetes mellitus and abnormalities of glucose homeostasis

- **Hemochromatosis**
- **Glycogen storage diseases**
- **Autoimmune biliary disease**

Hepatic fat accumulation is a well-recognized complication of diabetes with a reported frequency of 40–70%. Unfortunately, associated obesity is a frequently occurring confounding variable. Type 1diabetes is not associated with fat accumulation if glycemia is well controlled, but type 2 diabetes may have a 70% correlation regardless of blood glucose control.[1]

Nonalcoholic fatty liver disease refers to a broad spectrum of liver disease ranging from steatosis (bland fatty infiltration of hepatocytes) to nonalcoholic steatohepatitis (steatosis plus inflammation, necrosis, or fibrosis) to cirrhosis and, in some patients, to end-stage liver disease and hepatocellular carcinoma. These facts have been documented in studies done by Lee R .G et al (1989)[8] and Powell E .E et al [9](1990)

In 1980, Ludwig and colleagues coined the term Non Alcoholic Fatty liver Disease (NAFLD) and Non Alcoholic Steato- Hepatitis (NASH). Many names have since been proposed for this condition but this terminology has been widely accepted worldwide.

NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease is an increasingly recognized condition that may progress to end-stage liver disease. The pathological picture resembles that of alcohol-induced liver injury, but it occurs in patients who do not abuse alcohol.[2],[3] A variety of terms have been used to describe this entity, including fatty-liver hepatitis, nonalcoholic Laënnec's disease, diabetes hepatitis, alcohol-like liver disease, and nonalcoholic steatohepatitis. Nonalcoholic fatty liver disease is becoming the preferred term, and it refers to a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis.

The clinical implications of nonalcoholic fatty liver disease are derived mostly from its common occurrence in the general population and it’s potential to progress to cirrhosis and liver failure. Nonalcoholic fatty liver disease should be differentiated from steatosis, with or without hepatitis, resulting from secondary causes, because these conditions have distinctly different pathogeneses and outcomes.

The causes of FATTY LIVER with the exception of alcohol are:[4]

MACROVESICULAR (large fat droplets in hepatocytes)

Insulin resistance.

Syndrome X (obesity, diabetes, hypertriglyceridemia, hypertension.)

Lipodystrophy.

Dysbetalipoprotenemias.

Protein calorie malnutrition, Starvation.

Total parenteral nutrition, jejuno-ileal bypass

Rapid weight loss

Drugs: e.g. Methotrexate, Aspirin, Vitamin A, Glucocorticoids,

Amiodarone,Calcium Channel Blockers, Synthetic Estrogen And Nucleoside

Analogues

Inflammatory bowel disease

MICROVESICULAR (small fat droplets in hepatocytes)

Reye's syndrome

Acute fatty liver of pregnancy

Jamaican vomiting sickness

Drugs e.g. Valproic Acid, Tetracycline, Nucleoside Analogues

Environmental hepatotoxins (e.g. phosphorus, petrochemicals)

EPIDEMIOLOGIC FEATURES

RISK FACTORS

Obesity, Type 2 Diabetes Mellitus, and Hyperlipidemia are coexisting conditions frequently associated with nonalcoholic fatty liver disease. The reported prevalence of obesity in several series of patients with nonalcoholic fatty liver disease varied between 30 and 100 percent, the prevalence of type 2 diabetes varied between 10 and 75 percent, and the prevalence of hyperlipidemia varied between 20 and 92 percent.[2,5,6,7,8,9,10,11,12,13,14,15,16]

The prevalence of nonalcoholic fatty liver disease increases by a factor of 4.6 in obese people, defined as those with a Body-Mass Index of at least 30.[17] Regardless of body-mass index, the presence of type 2 diabetes mellitus significantly increases the risk and severity of nonalcoholic fatty liver disease.[18,19] Truncal obesity seems to be an important risk factor for nonalcoholic fatty liver disease, even in patients with a normal body-mass index.[20] About half of patients with hyperlipidemia were found to have nonalcoholic fatty liver disease on ultrasound examination in one study.[21] Hypertriglyceridemia rather than hypercholesterolemia may increase the risk of nonalcoholic fatty liver disease.[21]

Among people who are not obese and do not have diabetes, risk factors for NAFLD are impaired fasting glycemia, hypertriglyceridemia,

hyperuricemia, central obesity, hypertension and low levels of high-density lipoprotein (HDL) cholesterol.[22]

A family history of steatohepatitis or cryptogenic cirrhosis has also been implicated as a risk factor for this disorder.[23.] Nonalcoholic fatty liver disease may affect persons of any age and has been described in most racial groups. In most series, the typical patient with nonalcoholic fatty liver disease is a middle-aged woman, [2, 5, 6,7,8,13,16.] but some have found a higher prevalence of nonalcoholic fatty liver disease in males than in females.[24,25,,26,27.]

PREVALENCE

Nonalcoholic fatty liver disease affects 10 to 24 percent of the general population in various countries. The prevalence increases to 57.5 percent[26] to 74 percent[17, 27] in obese persons.

Nonalcoholic fatty liver disease is a common explanation for abnormal liver-test results in blood donors, and it is the cause of asymptomatic elevation of aminotransferase levels in up to 90 percent of cases once other causes of liver disease are excluded.[28]

Diabetes mellitus affects 7.8 percent of the U.S. adult population,[29] whereas about 50 percent (range, 21 to 78 percent)[30] of patients with diabetes have

nonalcoholic fatty liver disease. The association of diabetes and obesity may pose an added risk: among severely obese patients with diabetes, 100 percent were found to have at least mild steatosis, 50 percent had steatohepatitis, and 19 percent had cirrhosis.[31]

CLINICAL MANIFESTATIONS

Clinical Features

NAFLD is usually asymptomatic, although fatigue and discomfort in the right upper quadrant of the abdomen may be reported.[32.] The majority (56%–79%) of patients are overweight (body mass index [BMI] > 25 kg/m^2), and one-third have the metabolic syndrome.[33,34,35] Lean patients (BMI 25 kg/m^2) usually have at least one metabolic risk factor.[33] Hepatomegaly may be present, although signs of chronic liver disease are uncommon.[32.,36]

Hepatomegaly is the only physical finding in most patients. Acanthosis nigricans may be found in children with nonalcoholic fatty liver disease.[33,37] Findings of chronic liver disease and diminished numbers of platelets suggest that advanced disease with cirrhosis is present. A high proportion of patients with cryptogenic cirrhosis share many of the clinical and demographic features of patients with nonalcoholic fatty liver disease,[38]

suggesting that their cryptogenic cirrhosis is unrecognized nonalcoholic fatty liver disease.

Common symptoms and signs of 400 subjects with NAFLD (Data from the NAFLD clinic at Virginia Commonwealth University, previously unpublished data).[86]

Symptoms and signs	NAFLD (n=75) %	NASH (n=325) %
Asymptomatic	60	55
Fatigue	30	45
Pruritus	2	4
Right upper quadrant discomfort	30	32
Edema	4	5
Hepatomegaly	22	28
Stigmata of chronic liver disease	8	10
Obesity	65	60
Diabetes	45	50
Hypertension	60	65

LABORATORY ABNORMALITIES

Mildly to moderately elevated serum levels of aspartate aminotransferase, alanine aminotransferase, or both are the most common and often the only laboratory abnormality found in patients with nonalcoholic fatty liver disease. The ratio of aspartate aminotransferase to alanine aminotransferase is usually less than 1, but this ratio increases as fibrosis advances, leading to a loss of its diagnostic accuracy in patients with cirrhotic nonalcoholic fatty liver disease.[16] Serum alkaline phosphatase, γ-glutamyltransferase, or both are above the normal range in many patients, although their degree of elevation is less than that seen in alcoholic hepatitis.[6,7,13] An ALT or AST value >300IU/l should raise suspicion of alternate pathology.[40,2] The degree of abnormality is usually moderate and does not exceed 2–3 times the upper limit of normal values.[15,16] Unfortunately, none of these tests are sensitive or specific enough to establish a diagnosis of NAFLD with great accuracy

Other abnormalities, including hypoalbuminemia, a prolonged prothrombin time, and hyperbilirubinemia, may be found in patients with cirrhotic-stage nonalcoholic fatty liver disease.

Ferritin levels are increased in 20%–50% of patients, and elevated transferrin saturation (> 55%) is present in 5%– 10%.[41]

Autoantibodies are identified in 23%–36% of NAFLD patients and are associated with more advanced fibrosis.[42,43]

However, in studies of subjects with persistently elevated ALT values without an obvious explanation, NAFLD was found in only 70–80% of cases and 20–30% of subjects were found to have an alternate cause for their elevated liver enzymes [44],. Of note, 5.9% of subjects had a normal liver despite a complete evaluation.

IMAGING STUDIES

On ultrasonography, fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys. Regardless of the cause, cirrhosis has a similar appearance on ultrasonography. **Ultrasonography has a sensitivity of 89 percent and a specificity of 93 percent in detecting steatosis and a sensitivity and specificity of 77 percent and 89 percent, respectively, in detecting increased fibrosis.**[45] Fatty infiltration of the liver produces a low-density hepatic parenchyma on computed tomographic (CT) scanning. Steatosis is diffuse in most patients with nonalcoholic fatty liver disease, but occasionally, it is focal.

Sonography of fatty liver may be varied depending on the amount of fat and whether deposits are diffuse or focal.[46]

Diffuse steatosis may be:[47]

Mild: minimal diffuse increase in hepatic echogenicity; normal visualization of diaphragm and intrahepatic vessel borders.

Moderate: moderate increase in hepatic echogenicity; slightly impaired visualization of intrahepatic vessels and diaphragm.

Severe: marked increase in echogenicity; poor penetration of the posterior segment of right lobe of liver and poor or non visualization of the hepatic vessels and diaphragm.

Sonographic features of focal fatty change are:

Focal fat may show rapid change with time both in appearance and resolution, it does not alter the course or caliber of regional blood vessels, does not produce contour abnormalities, and the preferred site for both focal fat deposition and focal sparing is the area anterior to the portal vein at the porta hepatis. Some times focal fat may produce geographic map-like boundaries.

CT imaging of the liver provides a more specific method for the non-invasive diagnosis of NAFLD. Hepatic steatosis decreases the CT attenuation of the liver. When the hepatic parenchymal attenuation is 10 or more Hounsfield units lower than the spleen on a non-contrast-enhanced scan, a diagnosis of hepatic steatosis can be made. When intravenous contrast is administered, the hepatic enhancement lags behind the spleen and the liver-to-spleen attenuation differential exceeds 20 Hounsfield units.[48] While these features allow hepatic steatosis to be defined with a 76% positive predictive value [49],

Magnetic resonance spectroscopy allows a quantitative assessment of fatty infiltration of the liver,[50.] and a minimum of 5%–10% steatosis by weight is considered a requirement for the diagnosis of NAFLD.[39.]

The sensitivity of each imaging method increases with the degree of fatty infiltration, with at least 33% steatosis being optimal for detection.[49.]

HISTOLOGIC FINDINGS

Nonalcoholic fatty liver disease is histologically indistinguishable from the liver damage resulting from alcohol abuse. Liver-biopsy features include steatosis, mixed inflammatory-cell infiltration, hepatocyte ballooning and necrosis, glycogen nuclei, Mallory's hyaline, and fibrosis. The presence of these features, alone or in combination, accounts for the wide spectrum of nonalcoholic fatty liver disease. Portal tracts are relatively spared from inflammation, although children with nonalcoholic fatty liver disease may show a predominance of portal inflammation as opposed to a lobular infiltrate.[12] Mallory's hyaline is notably sparse or absent in children with nonalcoholic fatty liver disease.[12,33,37] In some patients with cirrhosis, the features of steatosis and necroinflammatory activity may no longer be present.[9,10]

A finding of fibrosis in nonalcoholic fatty liver disease suggests more advanced and severe liver injury. According to a number of cross-sectional studies including a total of 673 liver biopsies,[2,,5,,7,8,9,10,11,15,16,51,52] some degree of fibrosis is found in up to 66 percent of patients at the time of diagnosis, whereas severe fibrosis (septal fibrosis or cirrhosis) is found in 25 percent and well-established cirrhosis is found in 14 percent.

The combination of steatosis, infiltration by mononuclear cells or polymorphonuclear cells (or both), and hepatocyte ballooning and spotty necrosis is known as nonalcoholic steatohepatitis. Most patients with this type of nonalcoholic fatty liver disease have some degree of fibrosis, whereas Mallory's hyaline may or may not be present. The severity of steatosis can be graded on the basis of the extent of involved parenchyma .[53.] A system that unifies the lesions of steatosis and necroinflammation into a "grade" and those of the types of fibrosis into a "stage" has recently been proposed .[53.]

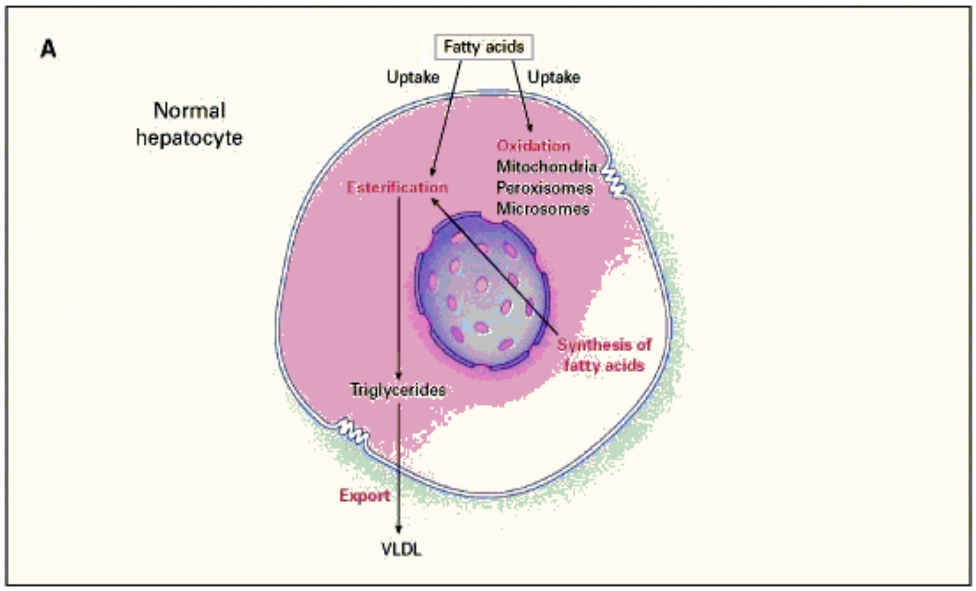
PATHOGENESIS [105]

The pathogenesis of nonalcoholic fatty liver disease has remained poorly understood since the earliest description of the disease. Much current thinking remains hypothetical, since the mechanism or mechanisms are still being worked out. It is not yet understood why simple steatosis develops in some patients, whereas steatohepatitis and progressive disease develop in others; differences in body-fat distribution or antioxidant systems, possibly in the context of a genetic predisposition, may be among the explanations.

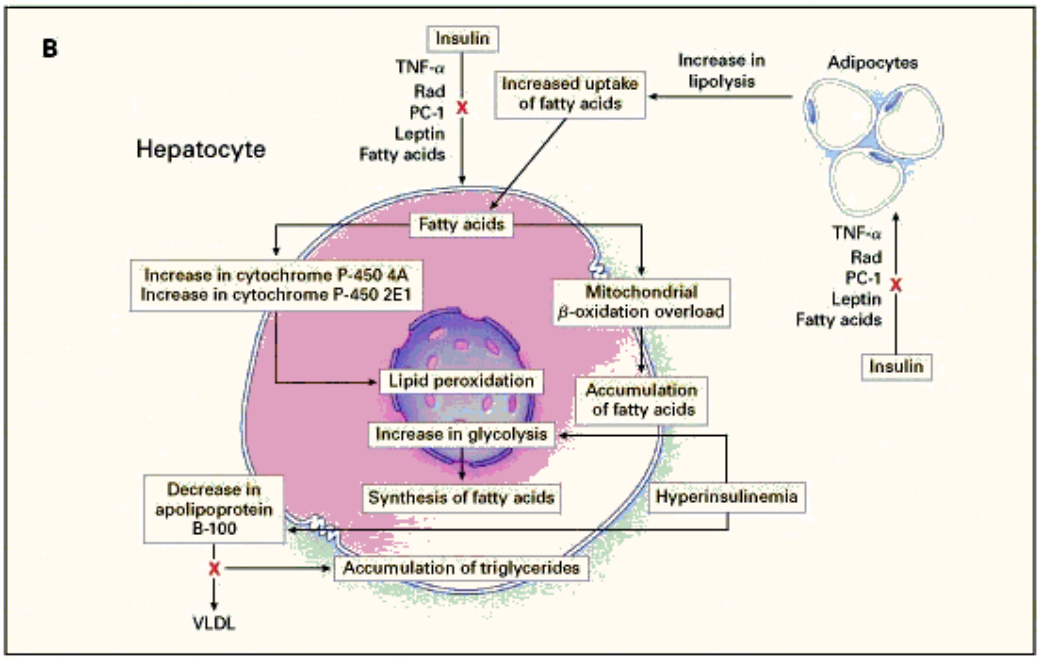
A net retention of lipids within hepatocytes, mostly in the form of triglycerides, is a prerequisite for the development of nonalcoholic fatty liver disease. The primary metabolic abnormalities leading to lipid

accumulation are not well understood, but they could consist of alterations in the pathways of uptake, synthesis, degradation, or secretion in hepatic lipid metabolism resulting from insulin resistance.

Possible Mechanisms of Pathogenesis of Nonalcoholic Fatty Liver Disease:



As shown in figure A, hepatic fatty acids are normally esterified into triglycerides, some of which are exported out of hepatocytes as very-low-density lipoproteins (VLDL). The increased level of lipids, mostly in the form of triglycerides, within hepatocytes in patients with **nonalcoholic fatty liver disease results from an imbalance between the enzyme systems that promote the uptake and synthesis of fatty acids and those that promote the oxidation and export of fatty acids.**



In figure B, **insulin resistance** (owing to inhibition of tumor necrosis factor α[TNF-α], Rad, PC-1, eptin, and fatty acids) **leads to the accumulation of fat in hepatocytes by two main mechanisms**: **lipolysis**, which increases circulating fatty acids, and **hyperinsulinemia**. Increased uptake of fatty acids by hepatocytes leads to mitochondrial β-oxidation overload, with the consequent accumulation of fatty acids within hepatocytes. Fatty acids are substrates and inducers of the microsomal lipoxygenases cytochrome P-450 2E1 and 4A.[54,55] The level of cytochrome P-450 2E1 is invariably increased in the liver of patients with steatohepatitis and may result in the production of free oxygen radicals capable of inducing lipid peroxidation of hepatocyte membranes.[54] Hyperinsulinemia resulting from insulin resistance increases the synthesis of fatty acids in hepatocytes

by increasing glycolysis and favors the accumulation of triglycerides within hepatocytes by decreasing hepatic production of apolipoprotein B-100.

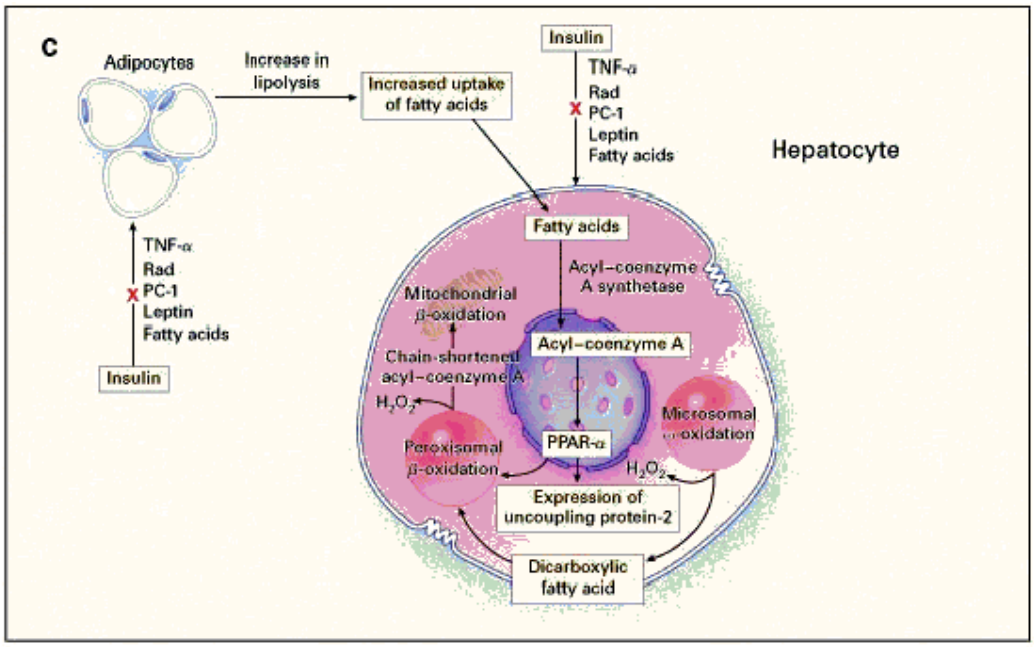
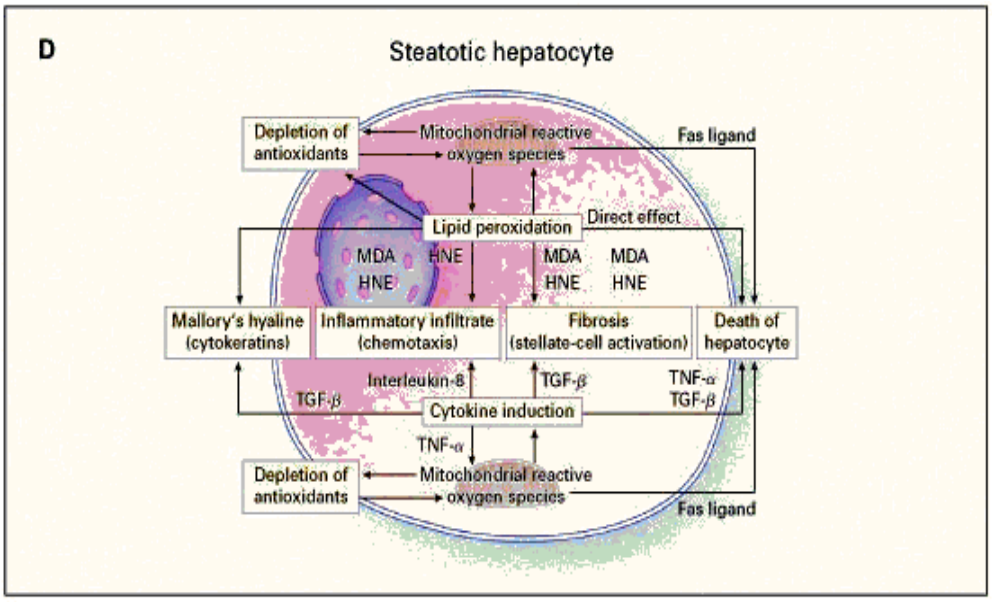


Figure C shows the relation between microsomal ω-oxidation, peroxisomal β-oxidation, and mitochondrial β-oxidation, as well as the regulatory role of peroxisome-proliferator–activated receptor α(PPAR-α) ligand. Microsomal ω-oxidation of fatty acids generates dicarboxylic fatty acids, which are further degraded by peroxisomal β-oxidation. Peroxisomal β -oxidation generates chain-shortened acyl–coenzyme A. Very-long-chain fatty acids are converted to acyl–coenzyme A by the action of acyl–coenzyme A synthetase. Acyl–coenzyme A serves as a substrate for peroxisomal oxidation, but if left unmetabolized, it functions as a PPAR-α ligand. PPAR-α controls the induction of genes involved in microsomal,

peroxisomal, and mitochondrial fatty-acid oxidation systems in liver, and it may also promote hepatic synthesis of uncoupling protein-2.[56] The role of this protein in the pathogenesis of nonalcoholic fatty liver disease remains uncertain. It may help inhibit hepatocyte apoptosis, but it may also increase the vulnerability of fatty hepatocytes to subsequent injury when exposed to secondary insults such as endotoxin or TNF-α.[56,57,32]



In figure D, **mitochondrial reactive oxygen species promote progression from steatosis to steatohepatitis** and fibrosis by three main mechanisms: lipid peroxidation, cytokine induction, and Fas ligand induction. Reactive oxygen species trigger lipid peroxidation, which causes cell death and releases malondialdehyde (MDA) and 4-hydroxynonenal (HNE).[58] MDA and HNE cause cell death; cross-link proteins, leading to the formation of Mallory's hyaline[59]; and activate stellate cells, promoting

collagen synthesis.[34] HNE has chemotactic activity for neutrophils, promoting tissue inflammation.[35] Reactive oxygen species also induce the formation of the cytokines TNF-α, transforming growth factor β(TGF-β), and interleukin-8. TNF-α and TGF-β cause caspase activation and hepatocyte death.[36,60] TGF-β activates collagen synthesis by stellate cells[34] and activates tissue transglutaminase, which cross-links cytoskeletal proteins, promoting the formation of Mallory's hyaline. Interleukin-8 is a potent chemoattractant for human neutrophils.[61] The TNF-α induced by reactive oxygen species further impairs the flow of electrons along the respiratory chain in mitochondria.[62] Mitochondrial reactive oxygen species can deplete hepatic antioxidants, allowing accumulation of more reactive oxygen species.[63,64] Mitochondrial reactive oxygen species cause expression of the Fas ligand in hepatocytes, which normally express the membrane receptor Fas.[65] The Fas ligand on one hepatocyte can then interact with Fas on another hepatocyte, causing fractional killing.

Insulin resistance is the most reproducible factor in the development of nonalcoholic fatty liver disease.[66] The molecular pathogenesis of insulin resistance seems to be multifactorial, and several molecular targets involved in the inhibition of insulin action have been identified. **Insulin resistance leads to fat accumulation in hepatocytes by two main mechanisms: lipolysis and hyperinsulinemia .**

Clinically significant amounts of dicarboxylic acids, which are potentially cytotoxic, can be formed by microsomal ω-oxidation. This pathway of fatty-acid metabolism is closely related to mitochondrial β-oxidation and peroxisomal β-oxidation. Deficiency of the enzymes of peroxisomal β-oxidation has been recognized as an important cause of microvesicular steatosis and steatohepatitis.[67] Deficiency of acyl–coenzyme A oxidase disrupts the oxidation of very-long-chain fatty acids and dicarboxylic acids, leading to extensive microvesicular steatosis and steatohepatitis. Loss of this enzyme also causes sustained hyperactivation of peroxisome-proliferator–activated receptor-α (PPAR-α), leading to transcriptional up-regulation of PPAR-α–regulated genes.[67] PPAR-α has been implicated in promoting hepatic synthesis of uncoupling protein-2, which is expressed in the liver of patients with nonalcoholic fatty liver disease.[56]

Increased intrahepatic levels of fatty acids provide a source of oxidative stress, which may in large part, be responsible for the progression from steatosis to steatohepatitis to cirrhosis. Mitochondria are the main cellular source of reactive oxygen species, which may trigger steatohepatitis and fibrosis by three main mechanisms: lipid peroxidation, cytokine induction, and induction of Fas ligand. Patients with steatohepatitis have ultrastructural mitochondrial lesions, including linear crystalline inclusions in megamitochondria.[68] This mitochondrial injury is absent in most patients

with simple steatosis and in healthy subjects.[69.] Patients with steatohepatitis slowly resynthesize ATP in vivo after a fructose challenge, which causes acute hepatic ATP depletion.[70] This impaired ATP recovery may reflect the mitochondrial injury found in patients with steatohepatitis.[68,69 .]

Thus, although symptoms of liver disease rarely develop in patients with fatty liver who are obese, have diabetes, or have hyperlipidemia, the steatotic liver may be vulnerable to further injury when challenged by additional insults. This has led to the presumption that **progression from simple steatosis to steatohepatitis and to advanced fibrosis results from two distinct events.**[71] First**, insulin resistance** leads to the accumulation of fat within hepatocytes, and second, **mitochondrial reactive oxygen** species cause lipid peroxidation, cytokine induction, and the induction of Fas ligand.

DIAGNOSIS

The diagnosis of nonalcoholic fatty liver disease is usually suspected in persons with asymptomatic elevation of aminotransferase levels, radiologic findings of fatty liver, or unexplained persistent hepatomegaly. The clinical diagnosis and liver tests have a poor predictive value with respect to histologic involvement.[72] Imaging studies, although of help in determining the presence and amount of fatty infiltration of the liver, cannot be used to accurately determine the severity of liver damage.

Liver biopsy is considered as best method for the detection of hepatic steatosis and it can also detect steatohepatitis.

The diagnosis of nonalcoholic fatty liver disease requires the exclusion of alcohol abuse as the cause of liver disease; a daily intake as low as 20 g in females and 30 g in males may be sufficient to cause alcohol-induced liver disease in some patients (350 ml [12 oz] of beer, 120 ml [4 oz] of wine, and 45 ml [1.5 oz] of hard liquor each contain 10 g of alcohol).[73,74,75] Other causes, such as viruses, autoimmune responses, metabolic or hereditary factors, and drugs or toxins, should be ruled out. The decision on how extensive the serologic workup should be must be individualized.

Even though liver biopsy is considered to be the best, some advocate that there are several drawbacks in using liver biopsy for this purpose. This

procedure is invasive, costly, and prone to complications, some minor, such as pain, others more severe with a recorded risk of death of 0.01%.[76,77,78] Notably, just as is the case in other chronic liver diseases, there is considerable sampling variability (40% for fibrosis staging), and a high intra and inter-pathologist variability.[79,80] Most importantly, the number of patients at risk for NAFLD is high enough that liver biopsy is not a practical and efficient tool for identifying those at risk of advanced fibrosis. Indeed an estimated 15 to 20% of the Western European population has steatosis [81] while more than half of Americans are overweight or obese.

So the diagnostic workup needs to be individualized and decisions taken accordingly.

Some newer methods are now emerging for the diagnosis of hepatic steatosis and steatohepatitis such as the H magnetic resonance spectroscopy And the fibrosure test for the detection of fibrosis. These tests may in course of time serve as a better noninvasive method for the detection of NAFLD.

NATURAL HISTORY

The natural history of nonalcoholic fatty liver disease is not well defined, but it seems to be determined by the severity of histologic damage. In five series, 54 of 257 patients with nonalcoholic fatty liver disease underwent liver biopsy during an average follow-up of 3.5 to 11 years.[8,9,10,11,52] Of these patients, 28 percent had progression of liver damage, 59 percent had essentially no change, and 13 percent had improvement or resolution of liver injury. Progression from steatosis to steatohepatitis[25,79] and to more advanced fibrosis[8,9,11,52] or cirrhosis[8,9,11,52] has been recognized in several cases. Some of the few deaths that occurred among the 257 patients were liver-related, including one from hepatocellular cancer. Thus, many patients with nonalcoholic fatty liver disease have a relatively benign course, whereas in some others, the disease progresses to cirrhosis and its complications.

Patients found to have pure steatosis on liver biopsy seem to have the best prognosis within the spectrum of nonalcoholic fatty liver disease,[11] whereas features of steatohepatitis or more advanced fibrosis are associated with a worse prognosis.[9,15,52] In one study,[52] progression of liver fibrosis occurred only in patients with necrosis and inflammatory infiltration on liver biopsy. In another study,[15] 36 percent of patients with nonalcoholic fatty liver disease died after a mean follow-up of 8.3 years; liver-related diseases were the second most common cause of death, exceeded only by cancer. There was a

trend toward more liver-related deaths among patients with steatohepatitis, which can be explained by the higher prevalence of cirrhosis among these patients.[15] Some data suggest that the coexistence of steatosis with other liver diseases, such as hepatitis C virus infection, could increase the risk of progression of the liver disease.[83] The natural history of cirrhosis resulting from nonalcoholic fatty liver disease has not been completely defined. In a recent study,[84] only 2.9 percent of 546 liver-transplantation procedures performed in a single center were for end-stage steatohepatitis.

This suggests that although nonalcoholic fatty liver disease is common, only a minority of patients will require liver transplantation.

TREATMENT

Many clinical trials are going on to find out an effective method of treatment of Nonalcoholic Fatty Liver Disease, many treatment options have been suggested and they are:

1. **Treatment of associated disorders**

Gradual weight loss, Control of diabetes, Control of dyslipidemia

2. Potential pharmacological approaches

Improved insulin resistance

Metformin, Thiazolidinediones: rosiglitazone, pioglitazone.

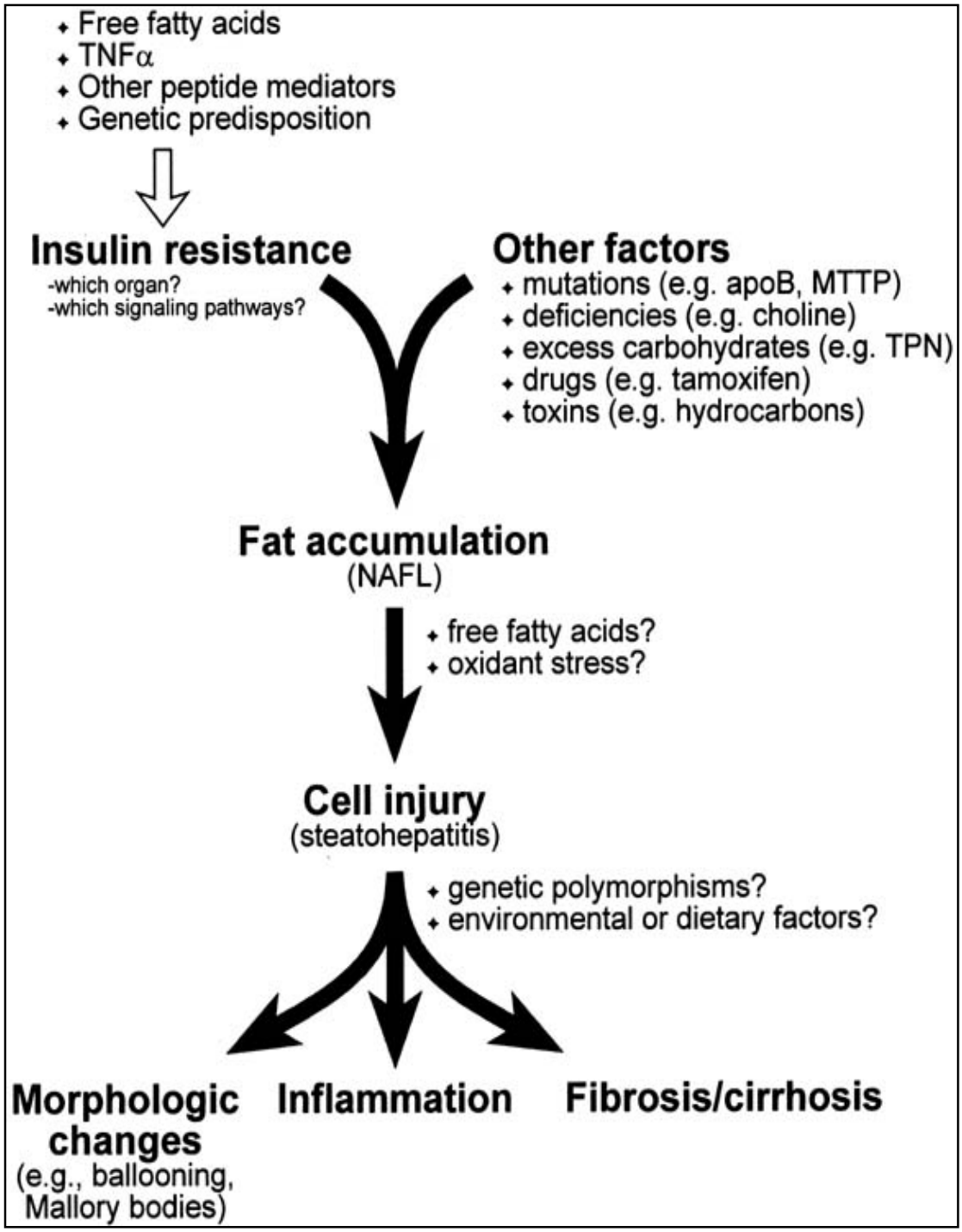
Improved dyslipidemia

Clofibrate, Gemfibrozil, Atorvastatin, Probucol.

Antioxidants

Tocopherol, Tocopherol/ascorbic acid, Betaine, Ursodeoxycholic acid, S-adenosylmethionine.

3. Liver transplantation



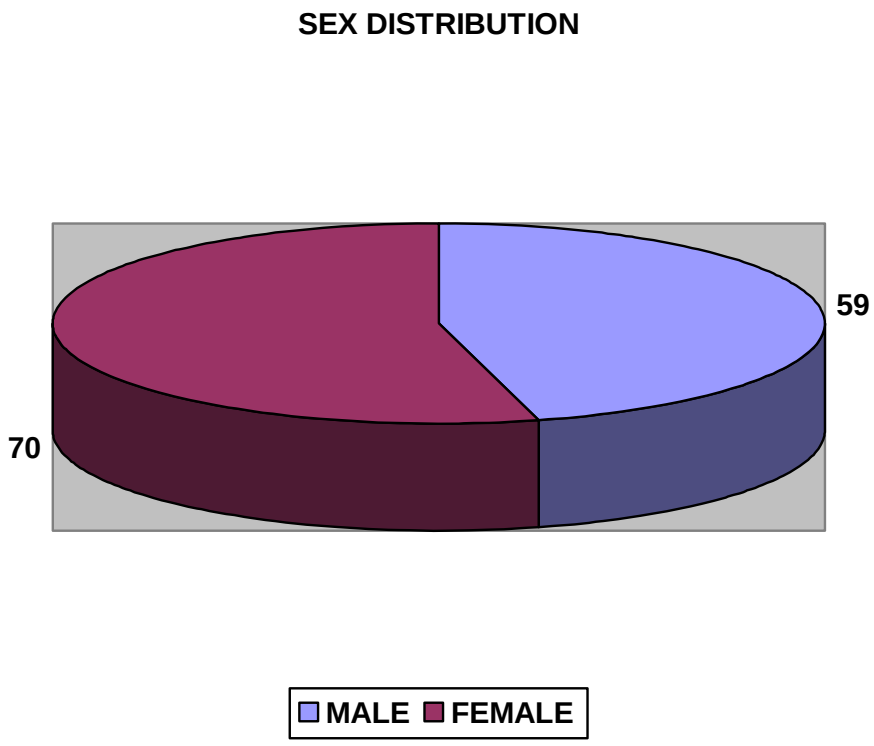
PATHOGENESIS OF NAFLD AND NASH- **"THE TWO HIT HYPOTHESIS"**

First hit causes development of fatty liver and the second hit results in inflammation.

RESULTS AND OBSERVATIONS

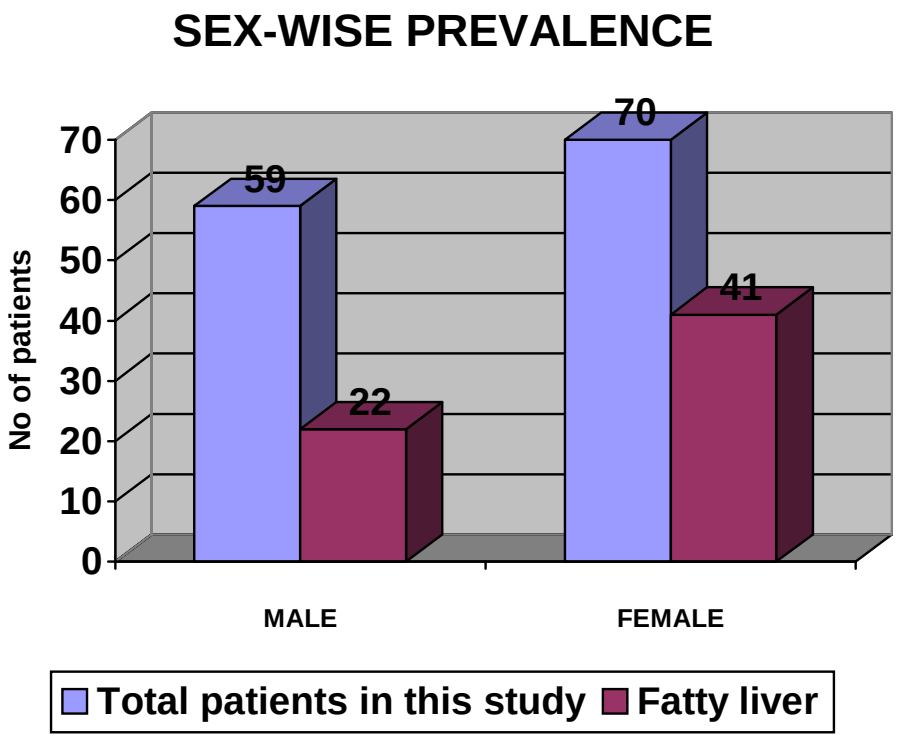
A total of 129 patients diagnosed with type 2 Diabetes mellitus for 3 years and more were included in this study after applying the selection criteria. Most of them belonged to the low and middle socioeconomic groups.

Out of the 129 participants 70 were females and 59 were males.



The age of the participants varied from 40 to 75 years and the mean age was 52.45 + or – 7.15 years.

Out of the total 129 participants 63 persons (48.8%) had ultrasonographically detected fatty liver. Most of them had moderate or severe steatosis ultrasonogram wise. They were called as the NAFLD (Non Alcoholic Fatty Liver Disease) group. Of these 63 persons 41 were females and 22 were males.



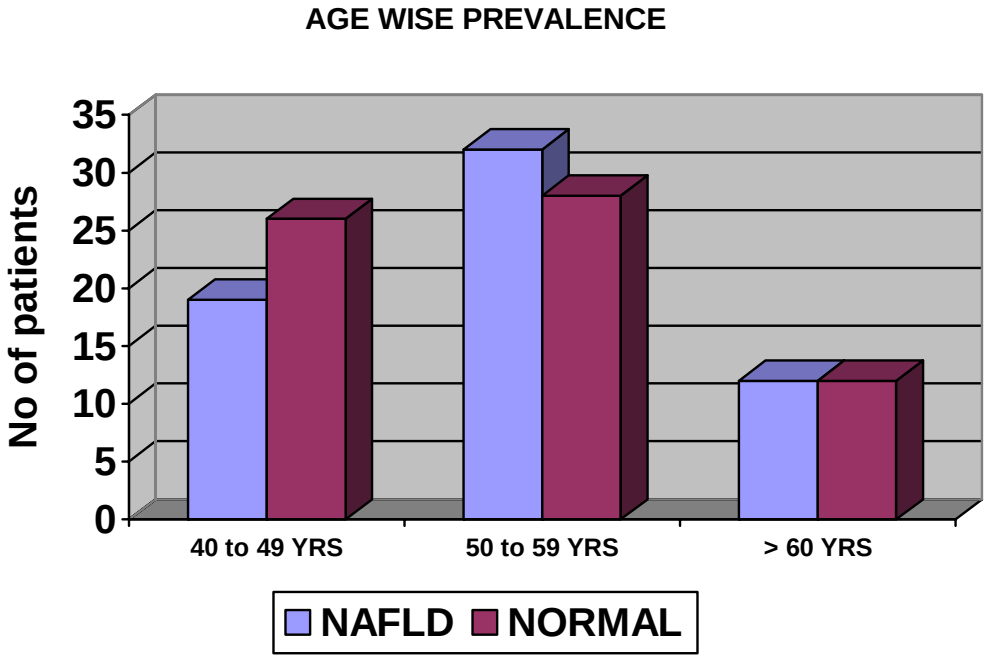
	Female(total 70)	Male (total 59)	P value
NAFLD in USG(63)	41 (58.57%)	22 (37.29%)	< 0.05
Normal USG(66)	29 (41.43%)	37 (62.71%)	

The duration of Diabetes varied from 3 to 20 years in the study group with a mean value of 5.48+ or – 3.57 years.

The mean duration of Diabetes in the fatty liver group was 5.47+ or – 3.19 years as compared to 5.48+ or – 3.94 years in the normal liver group. There was no significant difference between the NAFLD group and the normal group duration wise (P value >0.05).

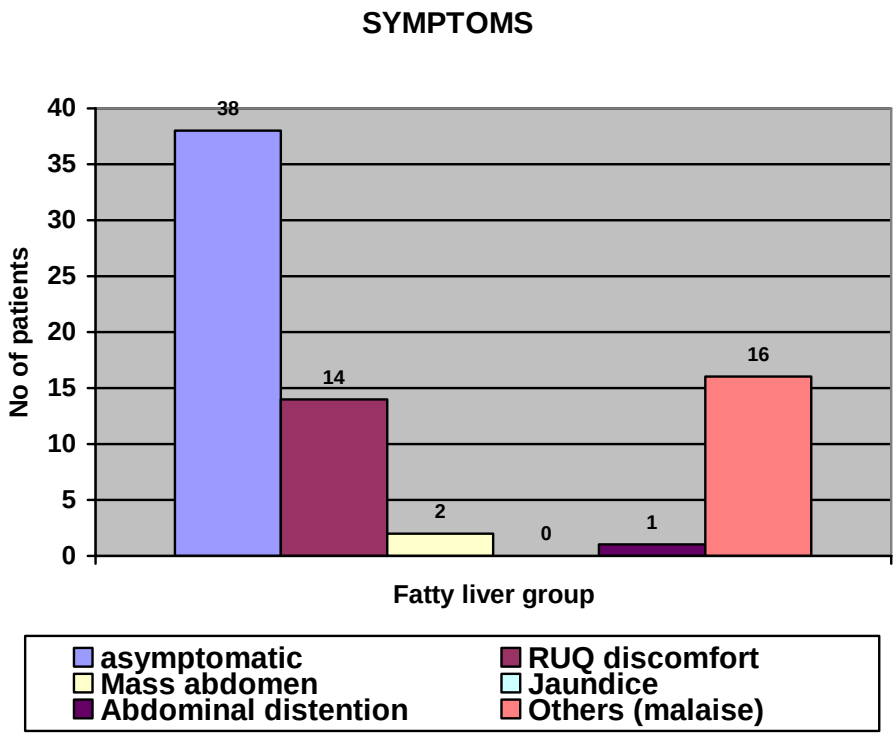
The age wise distribution of patients with and without fatty liver in Ultasonogram is as follows

Age group	Total (129)	NAFLD (63)	Normal (66)
40-49 years	45	19 (30.2%)	26 (39.4%)
50-59 years	60	32 (50.8%)	28 (42.4%)
> 60 years	24	12 (19.0%)	12 (18.2%)



CLINICAL FEATURES

Most of the persons with fatty liver were asymptomatic, i.e. 38 out of total 63. The next common symptom was right upper quadrant discomfort, which was present in 14 out of 63 patients, 2 persons in the fatty liver group had complaint of abdominal distension, and no patient had the complaint of jaundice, 16 persons had a feeling of generalized weakness and malaise.



Clinical examination of abdomen revealed hepatomegaly in 8 patients with fatty liver and 2 patients in the normal group. No patients in both groups had splenomegaly or ascites. Ultrasonography showed hepatomegaly in 12 out of 63 persons with fatty liver compared to 2 out of 66 persons in the normal liver group.

BODY MASS INDEX

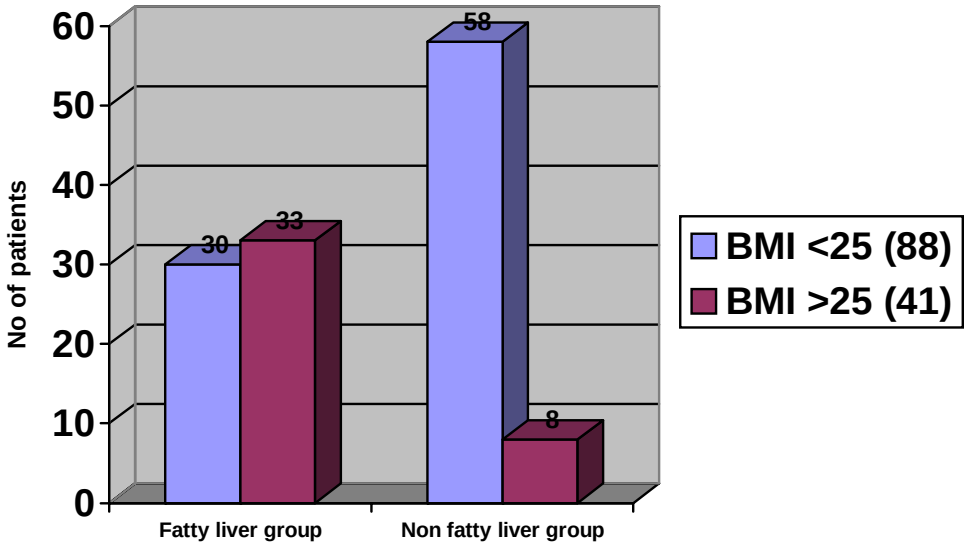
The Body Mass Index varied (BMI) from 17 to 37 kg/m^2 with a mean of a Body Mass Index of 23.60+or-3.17 kg/m^2.A BMI of 25 kg/m^2 was taken as a cut off between overweight and obese, 88 persons had a BMI below 25 kg/m^2 and 41 persons had a BMI of above 25 kg/m^2. **Only 4 persons had a BMI of more than 30 kg/m^2 all of them had fatty liver**. Out of the patients with a BMI of more than 25 kg/m^2 (total 41) 33 persons had fatty liver detected in ultrasonogram. In the low BMI group (total 88) 30 persons had Ultrasonographically detected fatty liver.

Mean BMI values:

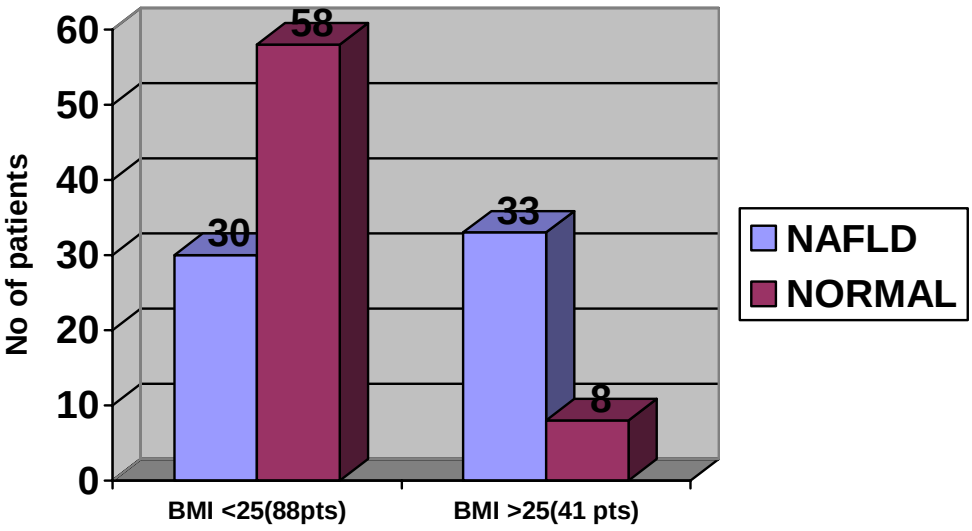
NAFLD group	Normal group	P value
24.97+ or _3.54 (kg/m^2)	22.29+ or _2.05(kg/m^2)	< 0.05

BMI (kg/m^2)	NAFLD GROUP(63)	NORMAL USG (66)
<25 (88)	30 (47.62%)	58(87.88%)
>25 (41)	33 (52.38%)	8 (12.12%)

BODY MASS INDEX



BMI



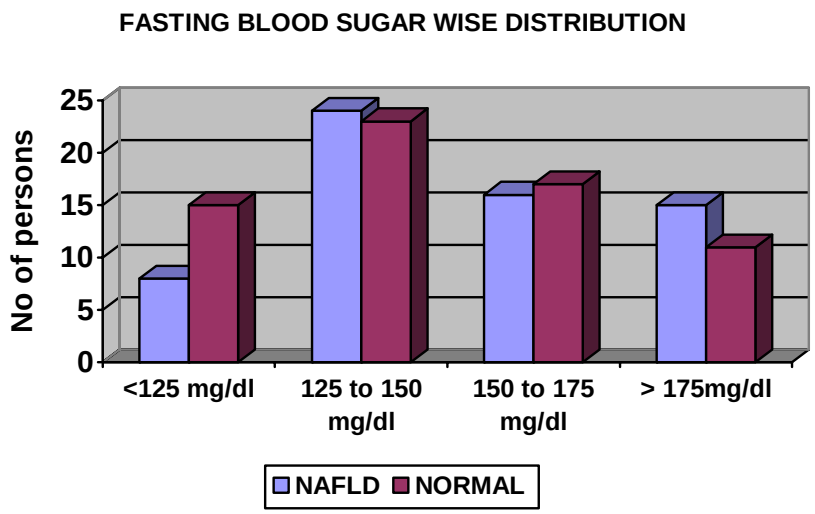
LABORATORY INVESTIGATIONS

BLOOD SUGAR

All the patients had a random, fasting and postprandial blood sugar estimation done.

The number of patients with and without fatty liver in the different fasting blood sugar categories is as follows.

FBS (mg/dl)	TOTAL	FATTY LIVER	NORMAL USG
< 125	23	8	15
125 TO 150	47	24	23
150 TO 175	33	16	17
>175	26	15	11



The mean fasting blood sugar in the above two categories are:

NAFLD group	Normal group	P value
156.19+ or _36.53mg/dl	146.67+ or _32.38mg/dl	> 0.05

LIVER FUNCTION TESTS

The liver function tests done included the Serum Transaminases, Serum Alkaline Phosphatase, Serum Total Bilurubin and Total Proteins

The normal values of serum transaminases was 5 to 35 IU/l. The normal value of Serum Alkaline Phosphatase is up to 150 IU/l. the participants were categorized into a low Transaminase level group of 25 IU/l or below and a high normal and increased Transaminase level group with a value of more than 25 IU/l.

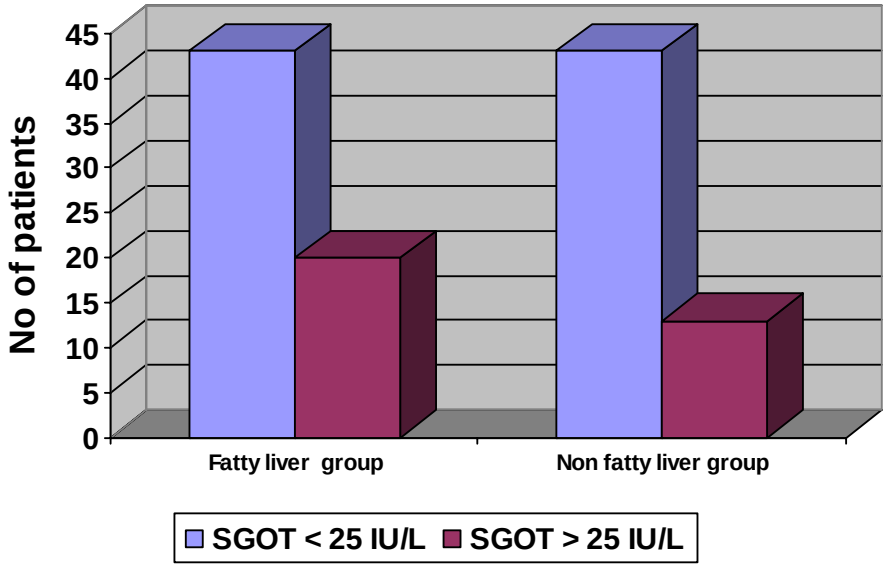
SGOT LEVELS:

SGOT levels	Total (129)	NAFLD group(63)	Normal group(66)
< 25 IU/L	96	40 (63.5%)	53 (80.3%)
25- 35 IU/L	20	11 (22.2%)	9 (13.6%)
> 35 IU/L	13	9 (14.3%)	4 (6.1%)

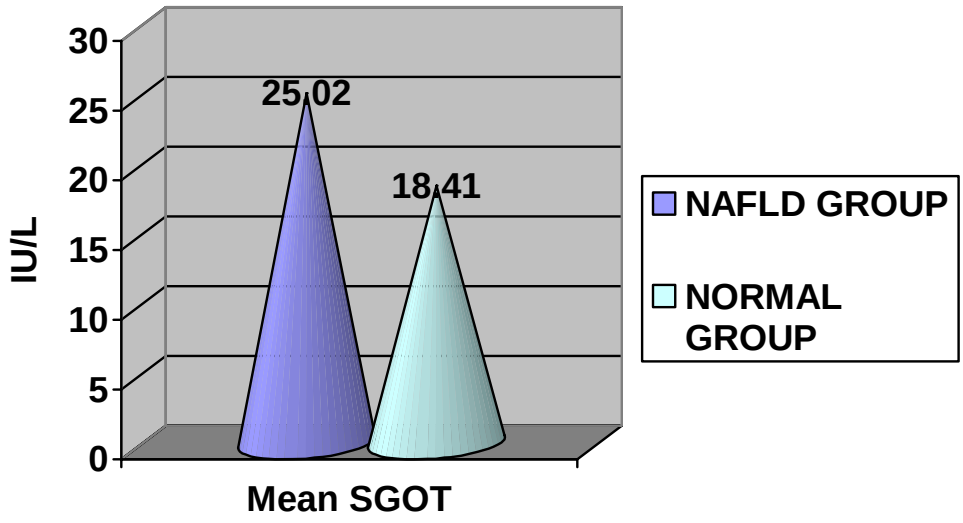
Mean SGOT values:

NAFLD group	Normal group	P value
25.02+ or _20.64 IU/L	18.41+ or _11.97 IU/L	< 0.05

SGOT VALUES:



MEAN SGOT LEVELS:



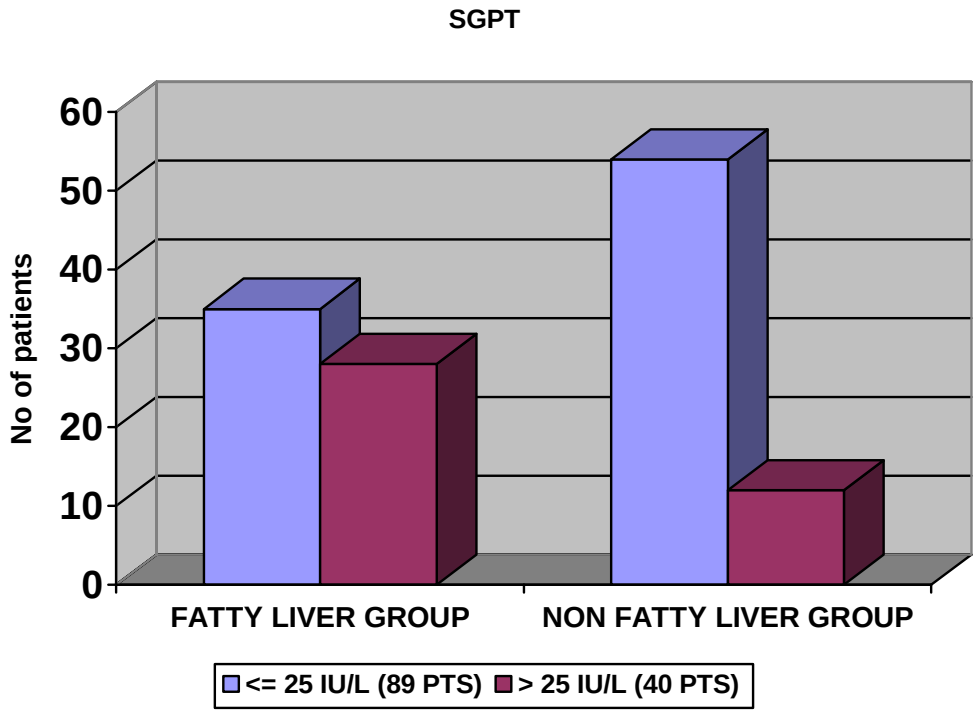
SGPT

Out of the total of 63 persons who had Ultrasonographically proven fatty liver 28 persons had an SGPT value of more than 25IU/L and 13 had an SGPT value of more than 35 IU/l.

Out of out of a total of 66 persons who had normal liver in Ultrasonography 12 persons had an SGPT value of more than 25IU/L and 3 persons had an SGPT value of more than 35 IU/l.

Mean SGPT Values:

NAFLD group	Normal group	P value
29+ or _28.35 IU/L	17.47+ or _10.02 IU/L	< 0.05



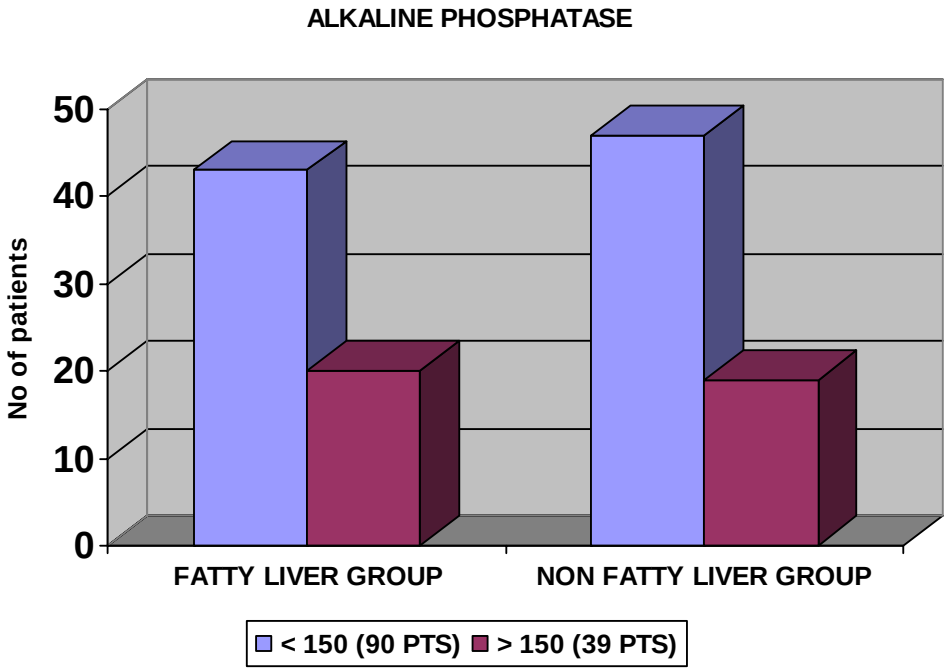
ALKALINE PHOSPHATASE

Out of the total of 63 persons who had Ultrasonographically proven fatty liver 20 (31.7%) persons had a Serum Alkaline Phosphatase value of more than 150 IU/L and 4 individuals had a value of more than 250 IU/l.

Out of a total of 66 persons who had normal liver in Ultrasonography 19 (28.78%) persons had a Serum Alkaline Phosphatase value of more than 150 and 1 person had a Serum Alkaline Phosphatase value of more than 250 IU/l.

Mean Alkaline phosphatase values:

NAFLD group	Normal group	P value
123.97+ or _66.13 IU/L	106.52+ or _68.75 IU/L	> 0.05



SERUM BILURUBIN:

The mean Serum total biluribin in the NAFLD group was 1.10 mg/dl and the Serum total Bilurubin in the normal group was 0.84 mg/dl. There was no significant difference in levels of Serum bilurubin between the two groups.

TOTAL PROTEINS:

The mean value of Total Protein in the NAFLD group was 6.43 gm and in the normal group it was 6.48 gm.There was no significant difference in levels of Total Protein between the NAFLD and normal liver groups.

FASTING LIPID PROFILE

The lipid done after an overnight fast of 12 hours included Total Cholesterol, Serum Triglycerides (TGL), Serum High Density Lipoprotein (HDL) and the Low Density Lipoprotein (LDL) value was calculated using the Friedwald formula.

According to the ATP III guidelines for the classification and treatment of lipid disorders, the levels of lipoproteins were considered abnormal if total cholesterol was above 200, if serum triglyceride level was above 150 mg/dl, serum HDL level was below 50 and LDL levels were above 100.

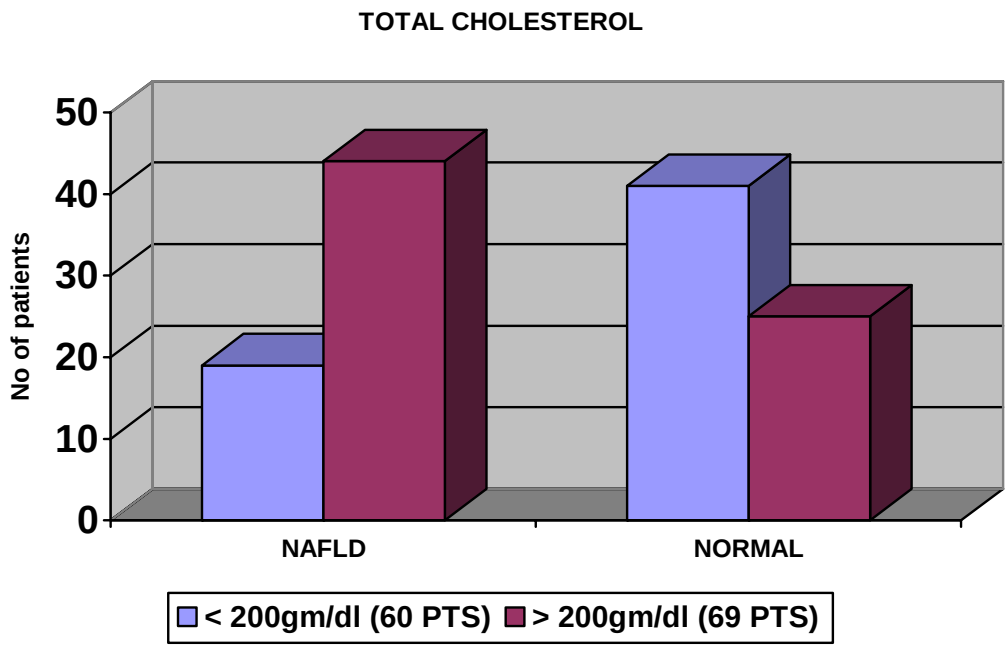
TOTAL CHOLESTEROL:

A total of 69 out of 129 had a high Total Cholesterol value. Among the NAFLD group out of the total 63 patients 44 (69.84%) had a Total Cholesterol value of more than 200 and among the normal liver group 25 (37.88%) out of the 66 had a Total cholesterol value of more than 200.

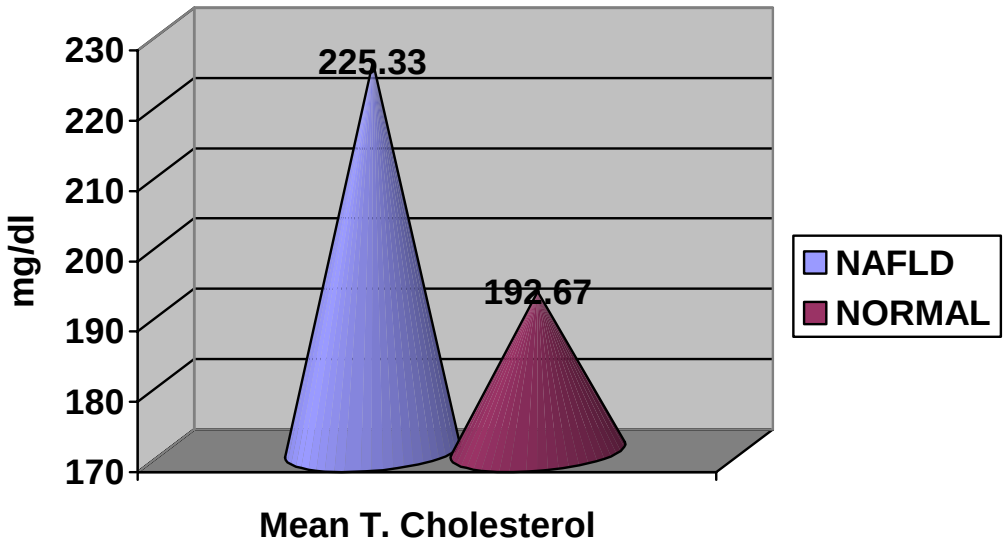
The mean total cholesterol values are as follows:

NAFLD group	Normal group	P value
225.33+ or _43.95 mg/dl	192.67+ or _35.38 mg/dl	< 0.05

TOTAL CHOLESTEROL VALUES



MEAN VALUES:

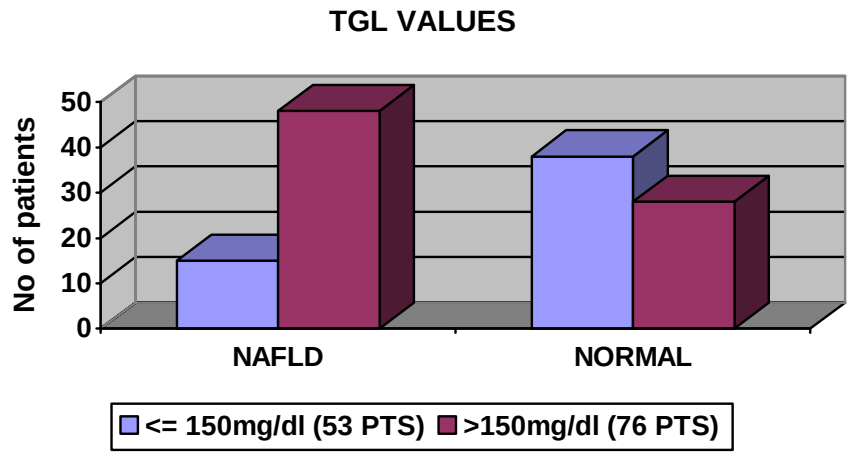


TRIGLYCERIDES

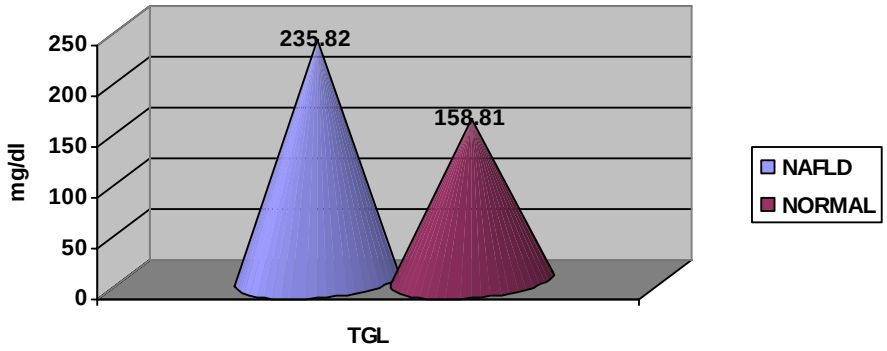
Out of the 63 patients in the NAFLD group 38 (60.32%) patients had a triglyceride level of more than 150 mg/dl of the 66 patients in the normal liver group 28 (42.42%) persons had a triglyceride level of more than 150 mg/dl.

The mean triglyceride levels:

NAFLD group	Normal group	P value
235.82 +or_ 105.18 mg/dl	155.81+ or _61.08 mg/dl	< 0.05



MEAN TGL VALUES:

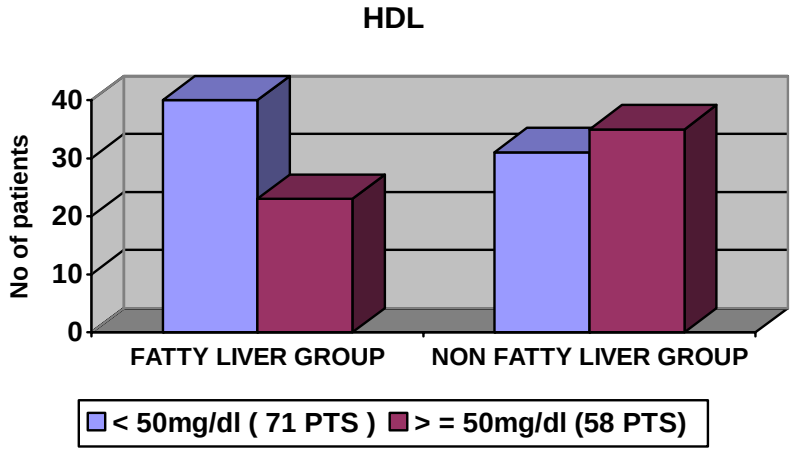


HDL

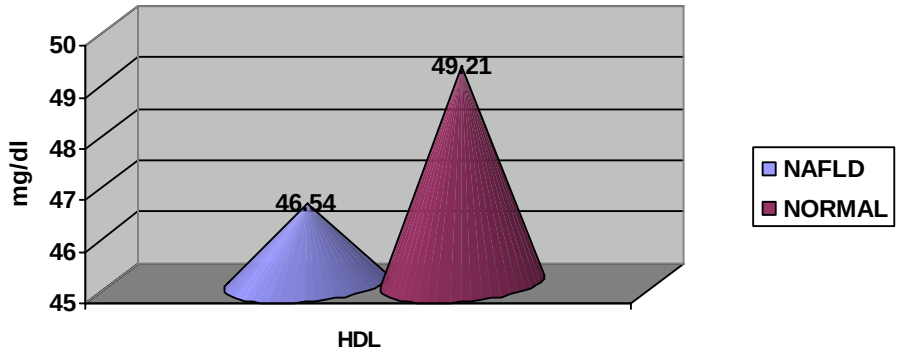
Out of the 63 patients in the NAFLD group 40 patients had a HDL level of less than 50 mg/dl of the 66 patients in the normal liver group 31 persons had a HDL level of less than 50 mg/ dl.

The mean HDL levels:

NAFLD group	Normal group	P value
46.24+ or _8.03 mg/dl	49.21+ or _9.93 mg/dl	> 0.05

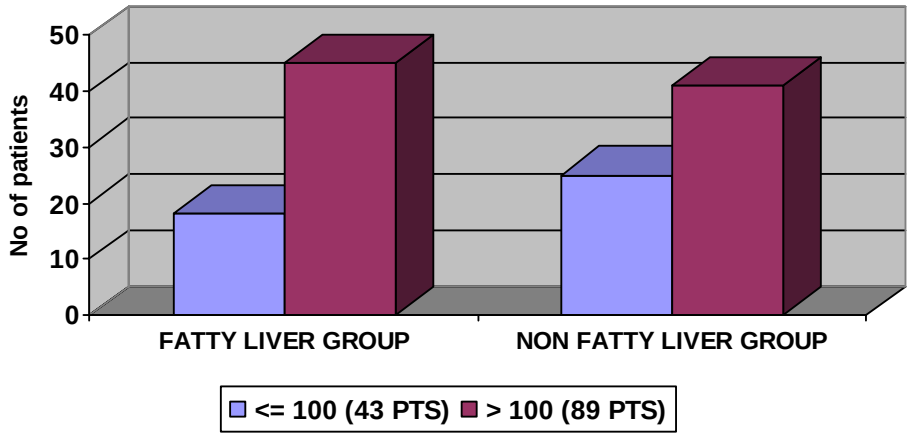


MEAN HDL VALUES:



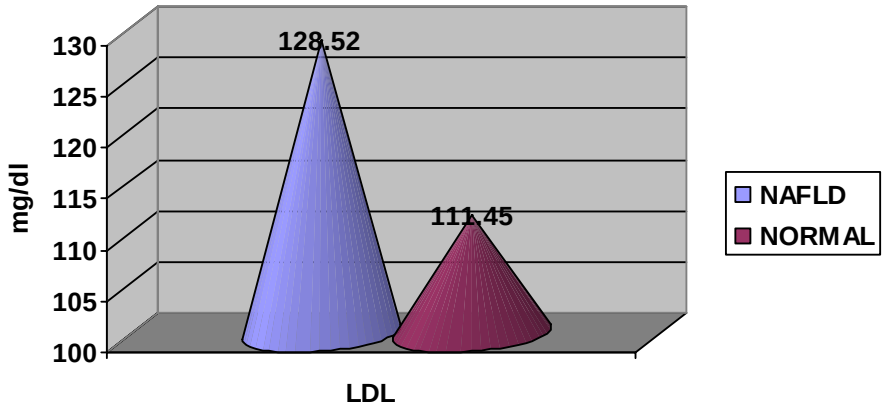
LDL

The LDL levels varied from 52 to 273mg/dl and a total of 86 patients had LDL levels above 100 mg/dl. In the fatty liver group 45 out of the 63 persons had elevated LDL values above 100 mg/dl. In the normal liver group 41 out of the 66 persons had an elevated LDL level of more than 100 mg/dl.



MEAN LDL VALUES:

NAFLD group	Normal group	P value
128.52+ or _41.66 mg/dl	111.45+ or _27.80 mg/dl	< 0.05



ABSTRACT OF STATISTICAL ANALYSIS

MEAN VALUES:

PARAMETER	**NAFLD GROUP (n=63)**	**NORMAL USG GROUP (n=66)**	**STATISTICAL SIGNIFICANCE AT 5% LEVEL**
DURATION OF DIABETES (yrs)	5.47 +or– 3.19	5.48 +or- 3.94	No significant difference (P value >0.05)
BMI (kg/m^2)	24.97 +or- 3.54	2.29 +or- 2.05	**Significant difference present (P value <0.05)**
SGOT (i u/l)	25.02 +or- 20.64	18.41 +or- 11.97	**Significant difference present (P value <0.05)**
SGPT (i u/l)	29.00+or-28.35	17.47+or-10.02	**Significant difference present (P value <0.05)**
ALP (i u/l)	123.97+or-66.13	106.52+or-68.75	No significant difference (P value >0.05)
BILURUBIN (mg/dl)	1.10+or-1.12	0.84+or-0.36	No significant difference (P value >0.05)
TOTAL CHOLESTEROL (mg/dl)	225.33+or-43.95	192.67+or-35.38	**Significant difference present (P value <0.05)**
TGL (mg/dl)	235.82+or-105.18	155.81+or-61.08	**Significant difference present (P value <0.05)**
HDL (mg/dl)	46.24+or-8.03	49.21+or-9.93	No significant difference (P value >0.05)
LDL (mg/dl)	125.82+or-41.66	111.45+or-27.80	**Significant difference present (P value <0.05)**

ABSTRACT OF DATA:

LIVER ENZYMES:

ENZYME LEVEL(IU/L)/ TOTAL	NAFLD GROUP(63)	NORMAL USG(66)	P VALUE
SGOT < 25(96)	43	53	>0.05
>=25(33)	20	13	
SGPT < 25(89)	35	54	**<0.05**
>=25(40)	28	12	
ALP <=150 (90)	43	47	>0.05
> 150 (39)	20	19	

LIPID PROFILE

PARAMETER(mg/dl)/ TOTAL	NAFLD GROUP (63)	NORMAL USG(66)	P VALUE
TC <=200 (60)	19	41	**<0.05**
>200 (69)	44	25	
TGL <=150 (53)	15	38	**<0.05**
>150 (76)	48	28	
HDL < 50 (71)	40	31	>0.05
>=50 (58)	23	35	
LDL <=100 (43)	18	25	>0.05
>100 (86)	45	41	

Statistically Significant difference at 5% level (P=0.05) between NAFLD and NORMAL USG groups was present for SGPT, Total Cholesterol and Triglyceride levels. Other parameters did not show any significant difference by comparing the two groups using ‘chi square test’. But mean LDL levels in the NAFLD group were much higher than in that of the normal group.

LIVER BIOPSY

Liver biopsy was done only in 7 selected cases (10 % of cases with NAFLD on USG) for the confirmation of diagnosis and to get a bird's eye view of the histological changes. Out of the 7 samples 1 patient had steatosis along with significant inflammatory cell infiltrate in the periportal regions with evidence of hepatocyte necrosis and feathery degeneration of hepatocytes signifying steatohepatitis. All the six others had steatosis only. No sample showed any evidence of cirrhosis or malignancy.

DISCUSSION

A total of 129 patients were included in this study after applying the selection criteria,

Out of the 129 type 2 diabetics included in this study 71 were females and 59 were males, the number of males was lesser than females because alcohol intake was taken as exclusion criteria and so many males got excluded.

of the 129 diabetics included in this study 63(48 %) of them had ultrasonographically detectable fatty liver, according to several reports the prevalence of fatty liver in Diabetes mellitus is more than that of the general population, many studies have shown that the prevalence of NAFLD in type 2 Diabetes mellitus was up to 70%.

The study of fatty liver in type 2 Diabetes mellitus

Series	Prevalence of NAFLD by USG (%)
Present (n=129)	48.8%
Daad H akbar (n=119)[85]	55%
Gupte P et al (n = 100)[108]	49%
Kelley D E et al (NA)	63%

The prevalence of fatty liver in this study group is similar to the prevalence observed in other studies.

Out of the 70 female type 2 diabetics 41(58.6%) had fatty liver detected by ultrasonography and out of the 59 male type 2 diabetics 22 (37.29%) had fatty liver. In this study female sex had a higher prevalence of fatty liver (M: F ratio is 1: 1.57).

Many studies have shown that female sex has a higher predisposition to the development of fatty liver in the general population. In other studies conducted among type 2 diabetics the prevalence was found to be more among females[2,5,6,9].

There was no significant variation in the mean age between the NAFLD group and the normal liver group. The mean age of the study population was higher because only persons above the age of 40 years were recruited into the study.

NAFLD has been described in different age groups, and even studies have been done in pediatric populations[33, 37].

DURATION OF DIABETES:

The mean duration of Diabetes in persons with NAFLD was 5.47+ or – 3.19 years and the mean duration of Diabetes in persons with Normal liver in USG was 5.47+ or – 3.94 years.

No statistically significant relationship was found between the presence of NAFLD and the duration of Diabetes. The results are similar to the study conducted in Saudi Arabia (Daad H Akbar et al)[85].

BODY MASS INDEX:

The mean Body Mass Index in the NAFLD group was significantly higher than that of the normal group. 41persons had a BMI of more than 25 kg/m^2 and out of them 33 (80.48%) had NAFLD.

In many studies Body Mass Index had a significant relationship with NAFLD, and Obesity had an association of 100 % with NAFLD in a few studies. In the study done by Daad H akbar et al in Saudi Arabia, Obesity was identified as an independent factor for the development of NAFLD[85].

The number of persons with a BMI of more than 30 kg/m^2 was less compared to studies done in other countries. This is probably due to the low and middle socioeconomic status of the study group. In our study group too the persons with high BMI had prevalence of fatty liver equal to that observed else where.

In one study it was said that Prevalence of NAFLD in patients with obesity or type 2 diabetes can be as high as 80-90% (Silverman et al, 1989[31], 1990[19]; Angelico et al, 2003[87]), although large studies are not available. In one study, liver biopsy of people with diabetes, obesity or dyslipidaemia found an 82% prevalence of NASH (Marchenisi et al, 2004)[88].

CLINICAL SYMPTOMS:

Following are the clinical symptoms observed in the group with NAFLD Compared to other studies:

Symptoms and signs	Present series (n=63)	Virginia series[86] (n=75)	Saudi series[85] (n=64)
asymptomatic	60%	60%	80%
Fatigue	25%	30%	NA
Right upper quadrant discomfort	22%	30%	17%
Jaundice	0%	NA	NA

One study had mentioned that approximately 25% of subjects with NAFLD also carry a diagnosis of chronic fatigue syndrome[89].

Our study group had a slight but statistically insignificant variation in the incidence of symptoms.

The natural history of NASH in Australia was followed in 42 patients for up to 21 years [9]. Upper abdominal pain was a common reason for presentation. Many studies have shown that A high proportion of patients (48% to 100%) have no symptoms of liver disease, and a small percentage (especially children [12,91]) have vague abdominal discomfort or pain in the right upper quadrant [91,10] or fatigue and malaise [8,10]

HEPATOMEGALY:

On clinical examination and Ultrasonogram wise 12 patients (19 %) out of 63 with NAFLD group had hepatomegaly. The incidence of hepatomegaly in different studies in diabetics is as follows.

Series	No of patients (%)
Lal et al (1971)[93]	10 out of 25 (38.5%)
Vaishnava (1970)[94]	28 out of 113 (24.7%)
Virginia series (1996)[86]	16 out of 75 (22%)
Saudi series (2003)[85]	56 out of 64 (88%)
Present series	12 out of 63 (19 %)

There were gross variations in the incidence of hepatomegaly between many groups.

None of the patients in the NAFLD group had splenomegaly or ascites.

Many studies have shown that The most common finding at initial presentation is asymptomatic hepatomegaly [6,8,9,10]

LABORATORY INVESTIGATIONS:

The prevalence of NAFLD was not significantly different among different levels of fasting sugar levels in our study.

Many studies have shown that the levels of blood sugar did not have any correlation with development of NAFLD. More over HbA1c estimation was done in the Saudi study[85], and there was no significant relationship between glycemic control and NAFLD.

TRANSAMINASES AND ALKALINE PHOSPHATASE:

There was no statistical difference between the two groups in terms of SGOT and Alkaline phosphatase elevation in terms of number of persons showing enzyme elevation. But when the mean enzyme values were compared the NAFLD group had a statistically significant higher value than the normal group.

Asyptomatic elevation of transaminases is one of the commonest reported and studied abnormality in NAFLD. The most frequently noted abnormality is a two- to threefold elevation of levels of ALT and AST in plasma [6,8,9,10,12,13]. Van Ness and Diehl [72] found that 19% of patients (17 of 90) who had had liver biopsy for evaluation of chronically elevated plasma levels of ALT and AST in contrast to 7% to 9% of all patients who had had liver biopsies for other reasons, had nonalcoholic steatosis or steatonecrosis[2,90].

Alkaline phosphatase levels are abnormal in fewer than half of patients, [8,10]. Another article has stated that Liver transaminases may be normal, or only marginally elevated (Mofrad et al, 2003)[92]. There is poor correlation between biochemistry, ultrasonography and histology, and the entire histological spectrum of NAFLD can be seen in individuals with normal transaminase values (Mofrad et al, 2003)[92].

Some studies have mentioned that Liver enzyme levels in NAFLD patients fluctuate, normal values being present in up to 78% of patients at any one time.[95,96] When levels are elevated, the increase is mild and often restricted to one or both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The AST:ALT ratio is usually less than 1, although it may reverse in the presence of cirrhosis.[97.]

In Type 2 Diabetics enzyme elevation was found in a total of 19% of cases in persons with NAFLD in the Saudi study. Our study too has results comparable to that study.

The SGOT: SGPT ratio in the NAFLD group in this study was 0.86.

In cases with NAFLD the SGOT: SGPT ratio is less than 1 according to literature. In two major studies [9,10], levels of ALT were noted to be higher than levels of AST, a pattern that contrasts with that seen in alcoholic hepatitis.

Although values < 1 suggest NAFLD, a ratio of > or = 2 is strongly suggestive of alcoholic liver disease

There was no statistically significant difference in the levels of bilurubin and total proteins between the two groups which were similar to the observations done elsewhere.

FASTING LIPID PROFILE:

Traditionally Total Cholesterol and Triglyceride values were found to be elevated in persons with NAFLD. Our study population consisted of type 2 diabetics and atherogenic dyslipidemias are common among diabetics.

The Total Cholesterol, TGL, values were significantly higher in terms of number of persons showing elevation and also in terms of the mean values in the NAFLD group. The number of persons showing elevated LDL was similar in both groups but the mean LDL was much higher in the NAFLD group.

Type of Lipid	% of persons	Mean value (mg/dl)
Increased TC	69.8%	225.3 + or – 43.95
Increased TGL	76.2%	235.82 +or – 105.18
Increased LDL	60.6%	128.52 + or – 8.03
Decreased HDL	71.4%	46.54 + or – 41.66

The HDL values were similar in both groups with mean values being marginally lower in the NAFLD group.

The values observed in other studies are as follows. Hyperlipidemia (hypertriglyceridemia, hypercholesterolemia, or both) is another common abnormality and has been reported in 20% to 81% of patients with NAFLD [2,6,7,8,9,10,13]. Dyslipidemia was present in 65% of the cases of NAFLD at the Virginia NAFLD clinic[86]. In another study, Hypertriglyceridemia and fatty liver: clinical diagnosis of fatty liver and lipoprotein profiles in hypertriglyceridemic patients with fatty liver, Most of the patients with fatty liver had hypertriglyceridemia.

Our population too showed a positive correlation of NAFLD with elevated lipid levels.

Ongoing research has now shown that Non alcoholic fatty liver disease has a broad clinical spectrum, different presentations, most of the research has shown that NAFLD has a stable course, some subsets of the NAFLD population might have a progression to severe forms of disease with inflammation termed steatohepatitis and a minority may end up in having cirrhosis. A significant proportion of patients previously thought to have cryptogenic cirrhosis share many of the clinical and demographic features of NAFLD, suggesting that the etiology of their cirrhosis may be unrecognized NAFLD (Powell et al, 1990[9];

Cadwell et al, 1999[38]; Poonawala et al, 2000[98]). Outcomes of NAFLD are different among different groups and other studies that looked at the outcome of people with NAFLD and Diabetes also report a more aggressive form of disease and higher overall mortality and mortality related to liver disease (Sargin et al, 2003). Older age, increasing obesity, type 2 Diabetes and hypertriglyceridaemia appear to be the strongest independent predictors of more advanced disease (Angulo et al, 1999[16]; Dixon et al, 2001[101]).

Follow up of patients with NAFLD has been discussed and monitoring patients with NAFLD is difficult because liver enzyme levels tend to improve regardless of whether liver fibrosis worsens or improves.[102] In addition, it may take several decades of monitoring before the development of complications is observed. Therefore, follow-up should be focused on patients who have risk factors for advanced disease.

LIMITATIONS OF THIS STUDY

Although ultrasound was sensitive for the detection of steatosis its accuracy was greater if more than 30% of the liver was affected by steatosis. This might lead to an under-estimation of prevalence. But several studies have been conducted with Sonography alone and our study was based on those lines.

Since our study population was derived from the patients attending outpatient clinic, liver biopsy was not feasible in all cases because many patients were not willing for invasive procedures or inpatient stay. So only a representative sample from the fatty liver group had a liver biopsy done. As in all imaging procedures observer error is expected and we tried to minimize this error by review of images by another radiologist.

Moreover certain investigations like insulin levels, C- peptide levels, Hb A1c, Transferrin saturation and ferritin levels could not be done in our setup. So we were unable to document hyperinsulinemia etc.

CONCLUSIONS

1. Non alcoholic fatty liver disease is common among the type 2 diabetic population of this region.(prevalence 48.8% of type 2 diabetics)
2. Female sex has a significantly higher prevalence of non alcoholic fatty liver disease as observed in other geographical regions. (M: F ratio is 1: 1.57).
3. The persons with a higher body mass index are at a greater risk of developing non alcoholic fatty liver disease (80.49% diabetics with a BMI > 25 kg/m^2 had ultrasonographically proven fatty liver)
4. Most of the cases of non alcoholic fatty liver disease are asymptomatic. (60.32%). Right Upper Quadrant discomfort and malaise are other symptoms.
5. Hepatomegaly was the commonest physical finding in Non alcoholic fatty liver disease (in19.04%). It was found to be present in varying incidences in other studies.
6. No significant relationship was observed between the age of patient, duration of diabetes, fasting blood sugar levels and the presence of non alcoholic fatty liver disease by ultrasound.
7. There was a significant difference in mean serum transaminase (SGOT, SGPT) levels between the normal and fatty liver groups with

the fatty liver group having higher values. But absolute elevation of transaminases above normal was not seen in many cases.

8. There was no significant relationship observed between serum alkaline phosphatase, total bilurubin, and total proteins and the presence of fatty liver by ultrasound.
9. Significantly high Serum Total Cholesterol, Triglyceride and Low Density Lipoprotein Levels Were Present in Persons with Fatty Liver.
10. No significant correlation was observed between low High Density Lipoprotein levels and the presence of fatty liver in ultrasound but marginally low mean High Density Lipoprotein values were present in the fatty liver group.

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PROFORMA

NAME:	AGE/SEX:
OCCUPATION:	I.P / O.P NO:

COMPLAINTS

Rt UPPER QUADRANT PAIN	ABDOMINAL MASS	JAUNDICE
ABDOMINAL DISTENSION	OTHERS:	

CLINICAL FINDINGS

HEPATOMEGALY	SPLENOMEGALY
OTHERS:	

ALCOHOL INTAKE:	YES	NO

H/O DRUG INTAKE

AMIODARONE	VALPROATE	+ CHANNEL BLOCKE
ANTICANCER DRUGS	GLITAZONES	
OTHERS:		
DURATION OF DIABETES:		

HEIGHT (*cm*):	WEIGHT (*kg*):	BMI:

LIVER FUNCTION TESTS		**BLOOD SUGAR**	
SGOT		RANDOM	
SGPT		FASTING	
Sr. ALK. PHOSPHATASE		POST PRANDIAL	
Sr. BILURUBIN		**ULTRASONOGRAM**	
Sr. TOTAL PROTEINS			
LIPID PROFILE			
Sr.T. CHOLESTEROL			
Sr. VLDL			
Sr. LDL			
Sr. HDL			
Sr. TRIGLYCERIDES			

LIVER BIOPSY:

CONCLUSIONS

1. Non alcoholic fatty liver disease is common among the type 2 diabetic population of this region.(prevalence 48.8% of type 2 diabetics)
2. Female sex has a significantly higher prevalence of non alcoholic fatty liver disease as observed in other geographical regions. (M: F ratio is 1: 1.57).
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8. There was no significant relationship observed between serum alkaline phosphatase, total bilurubin, and total proteins and the presence of fatty liver by ultrasound.
9. Significantly high Serum Total Cholesterol, Triglyceride and Low Density Lipoprotein Levels Were Present in Persons with Fatty Liver.
10. No significant correlation was observed between low High Density Lipoprotein levels and the presence of fatty liver in ultrasound but marginally low mean High Density Lipoprotein values were present in the fatty liver group.

BIBLIOGRAPHY

1.Gavin N. Levinthal, , and Anthony S. Tavill, : Liver Disease and Diabetes Mellitus Clinical Diabetes Vol. 17 No. 2 1999

2.Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434-438

3.Schaffner F, Thaler H. Nonalcoholic fatty liver disease. Prog Liver Dis 1986;8:283-298.

4. Harrisons principles of internal medicine,:16 th ed. 2005,Dennis L.Kasper, Eugene Braunwald, Anthony S.Fauci ,Stephen L. Hauser, Dan L. Longo, J.Larry Jameson. page 1870

5.Adler M, Schaffner F. Fatty liver hepatitis and cirrhosis in obese patients. Am J Med 1979;67:811-816.

6.Itoh S, Yougel T, Kawagoe K. Comparison between nonalcoholic steatohepatitis and alcoholic hepatitis. Am J Gastroenterol 1987;82:650-654.

7.Diehl AM, Goodman Z, Ishak KG. Alcohollike liver disease in nonalcoholics: a clinical and histologic comparison with alcohol-induced liver disease. Gastroenterology 1988;95:1056-1062.

8.Lee RG. Nonalcoholic steatohepatitis: a study of 49 patients. Hum Pathol 1989;20:594-598.

9.Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 1990;11:74-80.

10.Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 1994;107:1103-1109.

11.Teli MR, James OFW, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. Hepatology 1995;22:1714-1719

12.Baldridge AD, Peres-Atayde AR, Graeme-Cook F, Higgins L, Lavine JE. Idiopathic steatohepatitis in childhood: a multicenter retrospective study. J Pediatr 1995;127:700-704.

13.Pinto HC, Baptista A, Camilo ME, Valente A, Saragoca A, de Moura MC. Nonalcoholic steatohepatitis: clinicopathological comparison with alcoholic hepatitis in ambulatory and hospitalized patients. Dig Dis Sci 1996;41:172-179

14.George DK, Goldwurm S, MacDonald GA, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 1998;114:311-318.

15.Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-1419.

16.Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356-1362.

17.Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in northern Italy. Ann Intern Med 2000;132:112-117.

18.Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12:1106-1110.

19.Silverman JF, O'Brien KF, Long S, et al. Liver pathology in morbidly obese patients with and without diabetes. Am J Gastroenterol 1990;85:1349-1355.

20.Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. Diabetes 1998;47:699-713.

21.Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sci 2000;45:1929-1934.

22..Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, Lim SK, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch Intern Med* 2004;164:2169-75.

23.Struben VMD, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. Am J Med 2000;108:9-13.

24.Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Tani S, Goto M. Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med 1988;27:142-149.

25.Luyckx FH, Desaive C, Thiry A, et al. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Relat Metab Disord 1998;22:222-226.

26.Tominaga K, Kurata JH, Chen YK, et al. Prevalence of fatty liver in Japanese children and relationship to obesity: an epidemiological ultrasonographic survey. Dig Dis Sci 1995;40:2002-2009.

27.Oshibuchi M, Nishi F, Sato M, Ohtake H, Okuda K. Frequency of abnormalities detected by abdominal ultrasound among Japanese adults. J Gastroenterol Hepatol 1991;6:165-168.

28. Daniel S, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M. Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. Am J Gastroenterol 1999;94:3010-3014.

29.Harris MI, Flegal KM, Cowie CC, et al. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National Health and Nutrition Examination Survey, 1988-1994. Diabetes Care 1998;21:518-524.

30.Creutzfeldt W, Frerichs H, Sickinger K. Liver diseases and diabetes mellitus. Prog Liver Dis 1970;3:371-407.

31.Silverman JF, Pories WJ, Caro JF. Liver pathology in diabetes mellitus and morbid obesity: clinical, pathological and biochemical considerations. Pathol Annu 1989;24:275-302.

32.Faggioni R, Fantuzzi G, Gabay C, et al. Leptin deficiency enhances sensitivity to endotoxin-induced lethality. Am J Physiol 1999;276:R136-R142.

33. Rashid M, Roberts EA. Nonalcoholic steatohepatitis in children. J Pediatr Gastroenterol Nutr 2000;30:48-53

34Leonarduzzi G, Scavazza A, Biasi F, et al. The lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates transforming growth factor β1 expression in the macrophage lineage: a link between oxidative injury and fibrosclerosis. FASEB J 1997;11:851-857.

35.Curzio M, Esterbauer H, Dianzani MU. Chemotactic activity of hydroxyalkenals on rat neutrophils. Int J Tissue React 1985;7:137-142.

36.Higuchi M, Aggarwal BB, Yeh ETH. Activation of CPP32-like protease in tumor necrosis factor-induced apoptosis is dependent on mitochondrial function. J Clin Invest 1997;99:1751-1758.

37.Manton ND, Lipsett J, Moore DJ, Davidson GP, Bourne AJ, Couper RTL. Non-alcoholic steatohepatitis in children and adolescents. Med J Aust 2000;173:476-479.

38.Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664-669.

39.Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37:1202-19.

40]Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology. 1994;107:1103–1109.

41.Chitturi S, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, et al. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. Hepatology 2002;36:142-9.

42.Loria P, Lonardo A, Leonardi F, Fontana C, Carulli L, Verrone AM, et al. Non-organ-specific autoantibodies in nonalcoholic fatty liver disease: prevalence and correlates. Dig Dis Sci 2003;48:2173-81

43.Adams LA, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic fatty liver disease. *Am J* Gastroenterol 2004;99:1316-20

44.Mathiesen UL, Franzen LE, Fryden A, Foberg U, Bodemar G. The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. Scand J Gastroenterol. 1999;34:85–91

45.Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radiol 1991;43:26-31.

46.WilsonSR,Rosen IE, Chin-Sang HB, et al:Fatty infiltration of liver : an imaging challenge.J Can Assoc Radiol 1982; 33:227-232.

47..Diagnostic Ultrasound 3rd ed: Carol M. Rumack, Stephanie R. Wilson, J. William Charboneau, Jo-Ann M.Johnson; page 96-97.

48.Jacobs JE, Birnbaum BA, Shapiro MA, Langlotz CP, Slosman F, Rubesin SE, et al.. Diagnostic criteria for fatty infiltration of the liver on contrast-enhanced helical CT. AJR Am J Roentgenol. 1998;171:659–664

49.Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al.. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123:745–750.

50.Longo R, Pollesello P, Ricci C, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. J Magn Reson Imaging 1995;5:281-285.

51.Knobler H, Schattner A, Zhornicki T, et al. Fatty liver -- an additional and treatable feature of the insulin resistance syndrome. QJM 1999;92:73-79.

52.Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117-1123.

53.Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-2474.

54.Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology 1998;27:128-133.

55.Leclercq IA, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. J Clin Invest 2000;105:1067-1075.

56.Chavin KD, Yang SQ, Lin HZ, et al. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Biol Chem 1999;274:5692-5700

57.Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc Natl Acad Sci U S A 1997;94:2557-2562.

58.Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81-128.

59.Zatloukal K, Bock G, Rainer I, Denk H, Weber K. High molecular weight components are main constituents of Mallory bodies isolated with fluorescence activated cell sorter. Lab Invest 1991;64:200-206.

60.Inayat-Hussain SH, Couet C, Cohen GM, Cain K. Processing/activation of CPP32-like proteases is involved in transforming growth factor β1-induced apoptosis in rat hepatocytes. Hepatology 1997;25:1516-1526.

61.Yoshimura T, Matsushima K, Tanaka S, et al. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci U S A 1987;84:9233-9237.

62.Lancaster JR Jr, Laster SM, Gooding LR. Inhibition of target cell mitochondrial electron transfer by tumor necrosis factor. FEBS Lett 1989;248:169-174.

63.Watson AM, Poloyac SM, Howard G, Blouin RA. Effect of leptin on cytochrome P-450, conjugation, and antioxidant enzymes in the ob/ob mouse. Drug Metab Dispos 1999;27:695-700.

64.Sastre J, Pallardo FV, Llopis J, Furukawa T, Vina JR, Vina J. Glutathione depletion by hyperphagia-induced obesity. Life Sci 1989;45:183-187.

65.Hug H, Strand S, Grambihler A, et al. Reactive oxygen intermediates are involved in the induction of CD95 ligand mRNA expression by cytostatic drugs in hepatoma cells. J Biol Chem 1997;272:28191-28193.

66.Marchesini G, Brizi M, Morselli-Labate AM, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107:450-455.

67.Fan C-Y, Pan J, Usuda N, Yeldandi AV, Rao MS, Reddy JK. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase: implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. J Biol Chem 1998;273:15639-15645.

68.Caldwell SH, Swerdlow RH, Khan EM, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 1999;31:430-434.

69.Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183-1192.

70.Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 1999;282:1659-1664.

71.Day CP, James OFW. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-845.

72.Van Ness MM, Diehl AM. Is liver biopsy useful in the evaluation of patients with chronically elevated liver enzymes? Ann Intern Med 1989;111:473-478.

73.Bellentani S, Saccoccio G, Costa G, et al. Drinking habits as cofactors of risk for alcohol induced liver damage. Gut 1997;41:845-850.

74.Bird GL, Williams R. Factors determining cirrhosis in alcoholic liver disease. Mol Aspects Med 1988;10:97-105.

75.Becker U, Deis A, Sorensen TI, et al. Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. Hepatology 1996;23:1025-1029.

76.McGill DB, Rakela J, Zinsmeister AR, Ott BJ**:** A 21-year experience with major hemorrhage after percutaneous liver biopsy.
Gastroenterology 1990, 99**:**1396-400.

77.Cadranel JF, Rufat P, Degos F: Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF)**.**
Hepatology 2000, 32**:**477-81.

78.Poynard T, Ratziu V, Bedossa P: Appropriateness of liver biopsy**.**
Can J Gastroenterol 2000, 14**:**543-8.

79.Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T, for the LIDO Study Group: Sampling variability of liver biopsy in nonalcoholic fatty liver disease.
Gastroenterology 2005, 128**:**1898-1906.

80.Younossi ZM, Gramlich T, Liu YC, Matteoni C, Petrelli M, Goldblum J, Rybicki L, McCullough AJ: Non-alcoholic fatty liver disease: assessment of variability in pathologic interpretations.
Mod Pathol 1998, 11**:**560-565.

81.Bellentani S, Bedogni G, Miglioli L, Tiribelli C: The epidemiology of fatty liver. Eur J Gastroenterol Hepatol 2004, 16:1087-93.

82.Andersen T, Gluud C, Franzmann MB, Christoffersen P. Hepatic effects of dietary weight loss in morbidly obese subjects. J Hepatol 1991;12:224-229.

83.Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33:1358-1364.

84.Charlton M, Kasparova P, Weston S, et al. Frequency of nonalcoholic steatohepatitis as a cause of advanced liver disease. Liver Transpl 2001;7:608-614.

85. Daad H. Akbar, Abeer H. Kawther ;Nonalcoholic fatty liver disease in Saudi type 2 diabetic subjects attending a medical outpatient clinic: prevalence and general characteristics – Observations -Diabetes Care**,** Dec, 2003

86.Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis. Med Clin North Am. 1996;80:1147–1166.

87.Angelico F, Del Ben M, Conti R et al (2003) Non-alcoholic fatty liver syndrome: a hepatic consequence of common metabolic diseases. Journal of Gastroenterology and Hepatology 18: 588-94

88.Marchesini G, Bugianesi E, Forlani G et al (2004) Non-alcoholic steatohepatitis in patients cared in metabolic units. Diabetes Research and Clinical Practice 63: 143-51

89.Haller DL, Sargeant C, Luketic VA, Sanyal AJ. A behavioral analysis of nonalcoholic fatty liver disease. Hepatology. 2003;38:233A

90.Propst A, Propst T, Judmaier G, Vogel W. Prognosis in nonalcoholic steatohepatitis . Gastroenterology. 1995;108:1607

91**.** Moran JR, Ghishan FK, Halter SA, Greene HL. Steatohepatitis in obese children: a cause of chronic liver dysfunction. Am J Gastroenterol. 1983;78:374-7.

92.Mofrad P, Contos MJ, Haque M et al (2003) Clinical and histologic spectrum of non-alcoholic fatty liver disease associated with normal ALT values. Hepatology 37: 1286-92

93**.**Lal H.B; Bahl A.L; and Gupta P.R; Hepatic changes in diabetes mellitus; J.Ind Med Assn, 56-69,1971.

94.Vaishnava H. Liver and distuebance of carbohydrate metabolism, JAPI; 18:659, 1970.

95..Ipekci SH, Basaranoglu M, Sonsuz A. The fluctuation of serum levels of aminotransferase in patients with nonalcoholic steatohepatitis. J Clin Gastroenterol 2003;36:371.

96..Yano E, Tagawa K, Yamaoka K, Mori M. Test validity of periodic liver function tests in a population of Japanese male bank employees. J Clin Epidemiol 2001;54:945-51.

97.Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol 1999;94:1018-22.

98.Poonawala A, Nair SP, Thuluvath PJ (2000) Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case control study. Hepatology 32: 689-92

99.World Health Organization: Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva, World Health Org., 1999

100.Sargin M, Uygur-Bayramicli O, Sargin H, Orbay E, Yayla A (2003) Association of non-alcoholic fatty liver disease with insulin resistance: OGTT indicated in non-alcoholic fatty liver disease? Journal of Clinical Gastroenterology 37: 399-402

101.Dixon JB, Bhathal PS, O'Brien PE (2001) Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 121: 91-100

102.Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005;42:132-8.

103.Zargar AH, Bhat MH, Laway BA, Masoodi SR. Clinical and aetiological profile of early onset diabetes mellitus: data from a tertiary care centre in the Indian subcontinent. J Postgrad Med 2001;47:27-9

104.Outcome of cadaver kidney transplantation in 23 patients with type 2 diabetes mellitus; Ann Van Mieghem et al ;Nephrol Dial Transplant (2001) 16: 1686-1691

105**.** Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002;346:1221-31.

106. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br J Med Clin Res Ed. 1986;292:13-5.

107.Ricci C, Longo R, Gioulis E, Bosco M, Pollesello P, Masutti F, et al. Noninvasive in vivo quantitative assessment of fat content in human liver. J Hepatol. 1997;27:108-13.

108. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, Patel N, Madan A, Amarapurkar A, Hafeezunnisa. Non-alcoholic Steatohepatitis in Type 2 Diabetes Mellitus, J. Gastroenterol. Hepatol., 19, 854-858, 2004